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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. II. — THE MECHANISM OF THE MOTOR REACTIONS OF PARAMECIUM.[1]

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AS pointed out in No. I. of these Studies,[2] most of the reactions of Paramecium (as well as other Protozoa) are known only in their general features. The mechanism of the reactions, — that is, the motions of the cilia by which they are brought about, has been determined for Paramecium only in Ludloff's study[3] of electrotaxis and my own of thigmotaxis. It is the purpose of the present paper to supply the lack thus indicated by giving a detailed account of the mechanism of the motor reactions of Paramecium.

The far-reaching importance of an accurate knowledge of the exact mechanism of the reactions of Paramecium is apparent when we recall the sweeping conclusions that have been drawn from the results of studies on the reactions of this and other unicellular organisms. Theories of the mechanism of thermotaxis, geotaxis, and other reactions have been put forth, accompanied by deductions as to the remarkable sensitiveness of protoplasm to the various classes of reagents, and these theories have borne an important part in forming various prevalent conceptions in general physiology and in compara-

[1] Scientific Results of a Biological Survey of the Great Lakes, directed by Jacob Reighard, under the auspices of the U. S. Fish Commission, No. 1. (Published by permission of the Hon. George M. Bowers, Commissioner of Fisheries.)

[2] Jennings, H. S.: Journal of physiology, xxi, pp. 258–322.

[3] Ludloff: Archiv f. d. ges. Physiol., 1895, lix, p. 525.

tive psychology. They depend for their validity on whether the mechanism assumed for the reactions is the correct one, and for this assumption a basis of observation has usually been lacking. As will appear, some conclusions that have been deduced with the utmost confidence lose their validity when the actual mechanism of the reactions becomes clear.

It has become the fashion to refer many processes taking place in ontogeny to chemotaxis, or other reactions to stimuli, the matter apparently being considered as therewith definitely put at rest, as if such reference permitted no further analysis. It seems often to be forgotten that movements induced by chemotaxis must be accomplished by some means, some mechanism; and upon the character of this mechanism depends largely the interpretation to be given to the process. It may aid in recalling this neglected fact to set forth the roundabout method by which certain free cells succeed in gathering about a source of attractive stimulus.

A third standpoint, from which a knowledge of the general mechanism of reactions to stimuli should be of interest, is that of comparative psychology. The stage of mental development represented by unicellular organisms has, since the work of Verworn, been studied comparatively little, at least in any thorough manner, and the disconnected phenomena observed have lent themselves to interpretation by different authors in the most varied ways. Some hold that such organisms have a complex psychology containing nearly all the elements of which the psychic life of higher animals is made up, while others maintain that the observed phenomena are explicable on the simplest grounds; that such organisms are merely automata of the most limited capabilities, and that the activities which they show require little more than the property of irritability for their explanation. It may be hoped that an exact knowledge of the mechanism of the reactions of one of these organisms may throw further light on the question of the simplicity or complexity of the "psychic life of micro-organisms."

With these problems in mind, an examination will be made of the motor reactions of Paramecium. The organism to be studied, Paramecium caudatum,[1] is a unicellular animal belonging to the group of

[1] In the first of these Studies (*loc. cit.*) the organism used for experimentation is called Paramecium aurelia. I adopted this name because the organism has been always so called in the Jena Physiological Institute, where the work was done, and because my attention had not been called to the possibility of a mistake

ciliate infusoria, and is well known in every biological laboratory, living by thousands in vegetable matter decaying in water. It is somewhat cigar-shaped in form, having a narrow but blunt anterior end and a broad, sharp posterior end. From the anterior end a broad depression known as the oral groove runs obliquely along one side (the oral side) to the mouth, in the middle of the body. Near the opposite side (the aboral side) are the two contractile vacuoles. The entire surface of the body is covered with cilia, by means of which the animal moves. The length of the animal is about 0.2 mm.

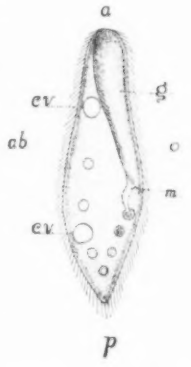

FIGURE 1. Paramecium caudatum. *a*, anterior end; *p*, posterior end; *ab*, aboral side; *o*, oral side; *m*, mouth; *g*, oral groove; *cv*, contractile vacuoles.

During its progress through the water, Paramecium comes in contact with sources of stimuli of various kinds; it strikes against mechanical obstructions, compelling it to turn aside, or it meets with various chemical substances in solution which either attract or repel it: heat, electricity, and other agents may likewise act as stimuli; to all these the animal reacts in a characteristic manner. The problem for solution is, exactly *how* is such a reaction accomplished? How does the attractive chemical compound succeed in affecting the locomotor organs of Paramecium so that it turns toward the source of stimulus? How does the repellent compound succeed in turning the Paramecium away from the source of stimulus?

In the first of these "Studies on Reactions to Stimuli" details were given as to the general features of the phenomena shown in the reactions of Paramecium. It was there shown that Paramecia are positively chemotactic to weak solutions of substances having an acid reaction, negatively chemotactic to substances having an alkaline reaction, and to many neutral salts. I shall give here in brief only enough of the gross appearance of these reactions to make intelligible the account of the finer mechanism and the way in which this mechanism is demonstrated.

in the name. But it appears that systematists make a distinction between Paramecium aurelia, with two micro-nuclei, and caudatum, with but one. As the Paramecia which I have been studying at Hanover, N. H., have but one micro-nucleus, I must call them P. caudatum. I believe them to be identical with the organisms used at Jena, though I did not investigate the micro-nucleus there.

The method by which the reactions of Paramecium toward most stimuli is demonstrated in practice is shown in Fig. 2. Paramecia are brought upon a glass slide and covered with a long cover-glass, supported near its two ends by bits of capillary glass tubing. The Paramecia swim at random throughout the preparation. Now a drop of any solution toward which the method of reaction is to be tested is introduced beneath the cover-glass by means of a capillary pipette, as shown in the figure. The Paramecia in their random swimming strike the edge of the drop, whereupon they react in a characteristic manner. If they are positively chemotactic to the substance in the drop — as they are if it is weakly acid in reaction — they soon gather in a dense swarm within the drop, as shown in Fig. 3. If, on the other hand, they are negatively chemotactic, the drop remains entirely empty, as shown in Fig. 4. This method is of course designed especially for the demonstration of chemotaxis. Thermotaxis, or the reaction toward heat or cold, may be demonstrated in a similar manner, or better by warming or cooling one end of the slide; still better by means of the apparatus devised by Mendelssohn.[1]

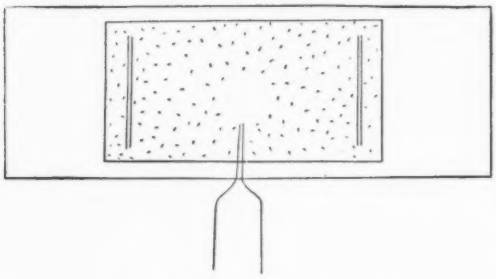

FIGURE 2. Method of testing the reactions of Paramecium.

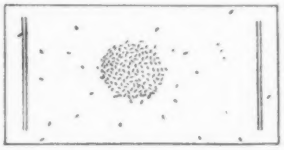

FIGURE 3. Paramecia collected in a dense swarm in a drop of a solution to which they are positively chemotactic.

Now, how does the substance in the drop in Fig. 3 succeed in affecting the cilia in such a way as to make the Paramecia turn toward and enter the drop?

Observation of the method by which the Paramecia collect in the drop shows that the foregoing question involves an assumption which is untrue. The Paramecia in the neighborhood of the drop do not turn toward it. The animals collect in the drop in an entirely dif-

[1] MENDELSSOHN: Archiv f. d. ges. Physiol., 1895, lx, p. 1.

ferent way. Suppose the drop to have been just introduced, as in Fig. 2. A Paramecium in its random course strikes by chance against the margin. It does not react, but keeps on its way across the drop until it comes to the other side (Fig. 5). There it reacts negatively, and turns back, swimming across the drop in another direction, till it again comes to the margin of the drop. There it again reacts negatively, and swims in a new direction, till it is turned back by the margin as before. This continues, so that the Paramecium is, as it were, imprisoned in the drop. Fig. 5 shows the course of a single Paramecium in such a drop. Other Paramecia enter the drop in the same way, purely by chance, and remain in the same manner, until the drop swarms with Paramecia. Owing to the restless hither and thither swimming of the animals on the slide, almost every one will in a short time have come by chance against the edge of the drop, will have entered and remained. Thus in a short time we get the appearance shown in Fig. 3; almost all the Paramecia in the preparation are collected in the drop. We say that the Paramecia are positively chemotactive to the substance in the drop, but so far as any motor reaction is concerned, evidently a more accurate statement is that after entering the drop by chance they are negatively chemotactic to the surrounding fluid.

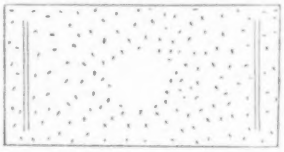

FIGURE 4. Negative chemotaxis toward a drop of sodic carbonate solution introduced 10 minutes before.

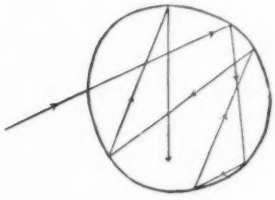

FIGURE 5. Course of a Paramecium in a drop of attractive substance.

The same thing may be shown to be true for thermotaxis or the reaction toward heat. In place of the chemical solution, we may introduce into a slide preparation of Paramecia in cold water a drop of water warmed to the temperature toward which the Paramecia appear to be positively thermotactic. We shall find that the Paramecia collect in the warm drop in exactly the same manner as in the case of the chemical compound just described: they enter by chance, then are negatively tactic to the surrounding cold water.

An extended examination of the reactions of Paramecium shows that this is typical for all apparently positive reactions. *In and of*

itself, considered as a motor reaction, there is no positive taxis in Paramecium. The so-called positive taxis always acts indirectly through (apparent) negative taxis. The question of how Paramecium turns toward an attractive substance may be thrown out, with the simple statement that Paramecium does not so turn.

There remains then only the mechanism of the negative reactions to be elucidated. We may first proceed to an examination of the method by which Paramecium succeeds in keeping out of a drop of some chemical to which it is negatively chemotactic, as in Fig. 4. For this purpose a drop of $\frac{1}{5}$ per cent sodium chloride solution may be selected. To understand the negative reaction, it is necessary to recall the animal's normal method of progression through the water. The unstimulated Paramecium swims nearly straight forward, though in a slightly sinuous course, the narrower, blunter end directed forward. At the same time it revolves on its long axis. Usually, but not invariably, this revolution seems to be from left to right, but there is much variation in the direction.

Now, when a Paramecium, swimming as above described, strikes the margin of the drop of sodium chloride solution, it reverses its course, darting straight backward, — the broad, pointed (posterior) end now being in the lead. At the same time, the direction of rotation on the long axis is reversed, the Paramecium revolving (usually) from right to left. Next the animal swings on its short axis, so as to bring the longitudinal axis of the body out of the line of direction in which it was first swimming. Then the infusorian begins to swim forward again, — following a course which lies at an angle to the course it was taking when it struck the drop of sodium chloride solution. Briefly stated, the animal has adopted the very rational course of backing off, turning in a different direction, then proceeding on past the obstacle, — much as any higher animal would have done.

We may here take up two questions, upon one of which we have already a certain amount of evidence, while the second is pressed upon us by the course of events in the reaction last described. The first is, — Do different kinds of stimuli have different methods of affecting the cilia? For example, does perhaps an acid cause the cilia to strike more strongly backward, driving the animal forward; an alkali, on the contrary, cause the cilia to strike more strongly forward, driving the animal backward? In this way, at first thought, the positive chemotaxis toward acids, the negative taxis toward alkalies might be ex-

plained. The results detailed thus far have shown, however, that such a view is not tenable for an explanation of positive chemotaxis, since the latter acts only indirectly through negative chemotaxis. Nevertheless, the general question still remains, — applicable, if not to acids and alkalies, perhaps to other chemical or physical agents. To determine the question with certainty, it will be necessary to immerse the Paramecia directly into solutions of various sorts, and observe the activities of the animals under these circumstances. At the same time evidence may perhaps be obtained on the second question referred to above, which is as follows: What causes the *turning away* of the Paramecium in the reaction toward sodium chloride, above described, after the backward course; and what determines the direction in which it turns? An obvious answer of course suggests itself, — the Paramecium turns away because it wants to get around the obstacle, and the direction in which it turns is determined by the position in which the object lies; it turns so as best to avoid the obstacle. This anthropomorphic view may perhaps be tested, and some evidence on our general question gained, by trying the effect of unlocalized stimuli, — stimuli that act upon the entire surface of the animal at once. This may of course be done by the method above mentioned, — complete immersion of the Paramecia in solutions of different kinds. There will then be no "obstacle" to get by, and, from the anthropomorphic standpoint, no excuse for turning in one direction or another.

With these considerations in mind, Paramecia are immersed directly in solutions of various sorts. This is done as follows: a solution is mixed in a watch-glass, then a quantity of water containing Paramecia is taken up with the pipette and injected energetically into the solution. They are quickly mixed thoroughly by the use of the pipette, then examined with the low power of the microscope.

The Paramecia may be first immersed in an alkaline solution, using for this purpose $\frac{1}{200}$ per cent sodium hydrate. Most of the Paramecia will be seen to swim energetically backward. This may be continued a few seconds or more, then many of the animals will be seen to turn and swim forward in a new direction. If a single specimen is watched, it may perhaps be seen to repeat the process of swimming backward, turning, and swimming forward several times; others may swim backward continuously.

Thus it appears that an alkali causes a reversal of the motion, followed by turning and swimming forward.

Let the Paramecia now be immersed in $\frac{1}{50}$ per cent acetic acid. As in the sodium hydrate, the Paramecia dart backward, only here the motion is more swift. The action of the acid seems much more energetic than that of the alkali; the Paramecia dart furiously backward for an instant, then turn quickly and rush forward for a moment, then perhaps backward again, then turn and rush forward. In a short time the backward motion has ceased; the Paramecia are all swimming forward at a furious rate, and soon they die. The difference in the action of the substances seems due to the fact that acids do not affect Paramecia at all, except when they are so strong as to be severely injurious; hence the great energy of the reaction and the quick death of the animals.

Thus acids also cause the infusoria to swim backward at first. The reaction is qualitatively the same for both acids and alkalies, only the intensity and duration of the different parts of the reaction varying.

In the same way, Paramecia may be immersed successively in a solution of some neutral salt, as $\frac{1}{5}$ per cent sodium chloride; in a solution that is effective only through its strong osmotic power, as a 10 per cent solution of cane sugar; in a watch-glass of water heated to 35° C.; in a watch-glass of water at the freezing temperature. In every case the characteristic features of the reaction are the same. The Paramecia swim backward for a longer or shorter time, then turn more or less, then swim forward. This may be repeated many times. In the different solutions the different features of the reaction may be more or less pronounced, in energy and duration, but the essential nature of the reaction is the same in all.

This series of experiments gives a definite answer to the first of the two questions proposed. Evidently different stimuli do *not* have qualitatively different methods of affecting the cilia. In every case the essential features of the reaction are the same, whatever the agent causing the reaction; only the intensity of the component activities varies. Every reagent causes at first a reversal of the motion of the animal, and therefore beyond doubt a reversal of the motion of the cilia. Since the same result is obtained with stimuli of so heterogeneous and opposite a character, it follows that the determining feature in this method of reaction must be internal. The Paramecium is like a machine in which the wheels are set for a certain motion; to every application of power from whatever source it responds by this motion. It is to be noted, moreover, that while in these experiments

the stimulus is continuous, the reaction is discontinuous, the motion being first backward, then sideways, then forward. The demonstration that a single stimulus does not have a single characteristic effect is therefore complete, since each stimulus causes three different motions of heterogeneous and even opposite character.

On the second question, as to what causes the turning away, and what determines the direction of turning, these experiments shed a certain amount of light, though they do not give a determinate answer. Since all these entirely unlocalized stimuli cause the turning away, it follows that the latter is not due to the localization of the stimulus: the Paramecia do not turn from nor toward the source of stimulus, — because they are completely surrounded by and immersed within it. Apparently the turning away is due, like the reversal, to an internal mechanism, not to any characteristic of the external stimulus. Neither can the *direction* in which they turn be determined by an external factor in these cases. It appears probable, therefore, that it is an internal factor that determines the direction of turning.

The next step is to try whether anything can be discerned in the structure of the Paramecium that will give a clue to the factor determining the direction of turning. The most obvious differentiations of the body of the animal are into anterior and posterior ends, and into oral and aboral sides, as shown in Fig. 1. The differentiation of the ends comes into consideration, of course, only in forward or backward motion. Has the differentiation into oral and aboral sides any relation to the turning movement? Owing to the continued rotation of the animal on its long axis, as it swims either backward or forward, it is impossible under ordinary conditions to observe whether there is any relation between this differentiation and the direction of turning. But a clue is given by the conduct of the animals when immersed in a solution of potassium iodide. This reagent has the property of almost indefinitely prolonging each of the component activities in the threefold reaction above described, and affords opportunity for a thorough analysis. When introduced directly into 1 per cent solution of potassium iodide the Paramecia begin, as in other reagents, to swim backward. But in potassium iodide solution this is continued six to eight minutes without change. After this they begin to turn, and this portion of the reaction is continued like the first, so that they spin around on the short axis of the body, as on a pivot, for half an hour at a time. Thus after ten minutes all the Paramecia in the watch-

glass will be whirling like tops. It is easy thus to have hundreds of specimens in the field at once with a low power lens, and of these many individuals will be in such a position that it is easy to determine the relation of the direction of turning to the two sides of the animal. It then appears that all are whirling toward the side that contains the contractile vacuoles, — that is, *toward the aboral side*. The direction of motion is indicated in Fig. 6. At first the Paramecia whirl as if on a pivot through the middle of the animal. Later the centre of rotation comes to lie outside the body of the animals, so that they now swim in circles. In every case the centre of the circle lies on the aboral side of the Paramecium, so that the animal is still turning toward the aboral side, though not in such short turns as at first.

FIGURE 6. Direction of motion of Paramecia whirling in 1 per cent solution of potassium iodide.

Thus under the stimulus caused by potassium iodide, the direction of turning is determined by an internal factor, — the differentiation into oral and aboral sides being the deciding feature. Taking this in connection with the fact that all unlocalized stimuli cause turning, it is a natural inference that the localization of a stimulus has no effect on the direction of turning. As this seems paradoxical and improbable on general principles, however, it must be tested by observation of the reactions of Paramecium toward stimuli that are localized. Is there a difference in the reactions of the animal according as the stimulus affects the anterior end, the oral side, the aboral side, or the posterior end? To determine the answer to this question, it is only necessary to introduce a drop of some chemical compound beneath the cover-glass of a slide preparation swarming with Paramecia, as shown in Fig. 2, and then to watch the edge of the drop with the low power of the microscope. Among the multitudes of Paramecia that strike the margin, many come against it obliquely, or touch it only on one side of the body, and their conduct is then easily observed. The reaction is the same in character as when the animal is immersed in a solution that acts as a stimulus; the Paramecium swims backward a distance, turns, and swims forward. It becomes evident at once that it *does not* turn directly away from the source of stimulus; the position of the drop seems to have little or no relation to the direction in which the Paramecium turns. Sometimes the animal

turns in such a way as to carry it away from the drop; in other cases it does not. There is no constancy in the direction of turning, and it is impossible to predict which way a Paramecium meeting the drop at a given angle will turn. In many cases the animal which at first brushed only the edge of the drop is carried, after turning, squarely against it, as illustrated in Fig. 7, in which the numerals indicate successive positions of the animal, while the arrows show the direction of motion. In such a case the whole operation is repeated; the animal backs off, turns in a new direction, and tries again. If the obstruction is large, the Paramecium may be thus compelled to try a dozen times before getting by the obstacle; such cases are often observed.

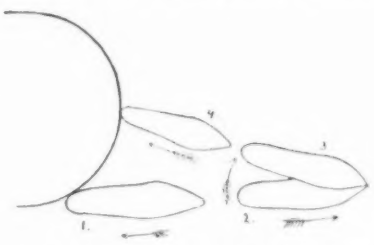

FIGURE 7. Paramecium striking a drop on one edge (1); swimming backward (2); turning (3); and swimming forward squarely against it (4).

This description applies equally well to the case of a mechanical obstruction, and to the reactions toward a very hot or very cold solution.

The inference previously made, that the localization of the stimulus has no relation to the direction in which the Paramecium turns, is therefore confirmed. It remains now to be decided whether the factor which determines the direction of turning when the stimulus is a solution of potassium iodide is the factor in other cases also. Does Paramecium always turn toward its aboral side, whatever be the nature and position of the stimulus?

To answer this question, it is necessary to use the method of observation in gelatine, devised by Jensen. Three grams of gelatine are dissolved by heat in 100 c.c. of ordinary water, and the Paramecia are observed in the thick solution. In this gelatine solution they are able to swim only very slowly, so that all the movements can be closely studied. Any chemical can be mixed with the gelatine solution, in order to observe the effects of unlocalized stimulation, or a drop of any solution can be introduced beneath the cover-glass with the capillary pipette, for observation of the effects of localized stimuli; in fact, the Paramecia may be studied exactly as in water, the only essential difference being that they move more slowly.

The Paramecia are therefore brought into such a gelatine solution,

and their conduct toward localized stimuli is observed. The unstimulated Paramecium swims straight forward, revolving slowly on the long axis. On coming in contact with a mechanical stimulus or a drop of some solution toward which it is negatively chemotactic, the animal swims backward very slowly, at the same time revolving slowly from right to left. Then it turns slowly, — *always toward the aboral side*. If the aboral side is above when the turning begins, the animal turns upward; if the aboral side is below, it turns downward; if to the right the animal turns to the right. This is true for a simple mechanical stimulus, for a chemical stimulus of any sort, or for a thermal stimulus; in fact, for any kind of a stimulus that can be tested in the gelatine. After some time in the gelatine solution the Paramecia are usually seen to be somewhat curved, and the curving is always toward the aboral side, as in Fig. 8. A similar curving may often be observed after a strong stimulus, when the Paramecia are mounted in water. The anterior end thus curved of course acts like the rudder of a boat in turning the course of the Paramecium toward the aboral side. Nevertheless, while it aids, it is evident that this is not the only factor in the turning, since Paramecia under other conditions are seen to turn sharply, or even to revolve almost as if on a pivot, when the body is not observably curved at all.

FIGURE 8. Paramecium curved toward the aboral side, under the influence of a powerful stimulus.

The invariable turning toward the aboral side may, as I have since discovered, be demonstrated, without the use of gelatine, in the following way: Paramecia intermingled with masses of bacterial zoöglœa are mounted on a slide and covered with a cover-glass, which is closely pressed down, so as to leave only a narrow space between slide and cover. It will usually be found that many Paramecia are imprisoned in small crevices in the bacterial zoöglœa, so that they can swim only from one end of the crevice to the other, and at each end they must turn. The cover-glass is so close to the slide that the animals can turn only in a horizontal plane. By watching the Paramecia as they turn at the end of the crevice, it will easily be seen that they invariably turn toward the aboral side.

We find then that the question last proposed is to be answered affirmatively. Paramecium after reversing *always turns toward the aboral side*, whatever be the nature and position of the source of stimulus.

A certain feature in the normal straightforward swimming of the unstimulated Paramecium appears to be connected with this fact. As has been often noted, the course in which an individual swims is not perfectly straight, but, as seen from above, the animal seems to swerve from side to side. This swerving has been figured by Verworn[1] and others. The actual path is a narrow spiral, the animal, of course, swerving up and down as well as from side to side. Now, observation of slowly moving individuals shows that at any point in this spiral course the aboral side is on the outside of the spiral. In other words, the Paramecium as it swims continually swerves toward the aboral side. As at the same time it rotates on its long axis, the position of the aboral side continually changes, so that the resulting path is a spiral one, and the general result is the same as if the Paramecium had progressed in a straight line. These facts are indicated in the accompanying diagram of the path of a Paramecium (Fig. 9). Apparently the method of reacting to a stimulus by turning toward the aboral side is a mere accentuation of the ordinary swerving toward that side.

FIGURE 9. Path of a Paramecium. The aboral side is everywhere directed toward the outside of the spiral.

It will here be necessary, in order to avoid misunderstanding, to give an account of an apparent exception to the rule that the Paramecium after stimulation always turns toward the aboral side. In my previous paper on the reactions of Paramecium (*loc. cit.*), I gave an account of the thigmotactic reaction of Paramecium; that is, the reaction due to continued contact with a solid body. I must here supplement the account by setting forth a little more fully certain features in that reaction.

On page 303 of my previous paper is figured a Paramecium resting with its anterior end against a solid body, and exhibiting the currents caused by the cilia under these circumstances, — that is, the currents characteristic of the thigmotactic reaction. As described in detail in the paper cited, the cilia in the oral groove at such a time beat strongly backward, driving a swift current of water toward the rear and causing a vortex to form on the oral side of the anterior half of

[1] VERWORN, M.: Psychophysiologische Protisten-Studien, 1889, p. 116.

the animal, as shown in Fig. 19 (*loc. cit.*), while the cilia on the remainder of the body are quiet, or possibly have varying motions tending to counteract the motion of translation that might be induced by the cilia in the oral groove. Meanwhile, the body of the animal remains at rest. This type of activity of the cilia is especially characteristic for resting Paramecia. But sometimes the solid body which causes the thigmotactic reaction is very small and movable, and so does not restrain in any way such motions of the animal as might naturally result from the activity of the oral cilia. In this case the Paramecium moves through the water in a peculiar way, carrying with it the small particle that has induced the thigmotactic reaction.

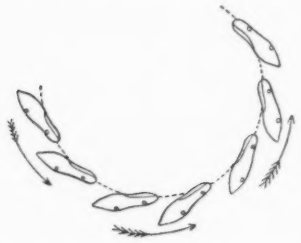

FIGURE 10. Motion of Paramecium in the thigmotactic reaction to a movable particle.

"The cilia beating strongly backward in the oral groove of course impel the animal forward. But as the active cilia are all on one side, there is also a tendency to move toward the opposite side. The resultant of these two motions at right angles to each other is a motion in the circumference of a circle. In fact, the animal moves forward in exactly the lines indicated by the arrows in Fig. 19, only, of course, in the opposite direction from the water currents. The ciliary motion is thus the same whether the thigmotactic animal is at rest or in motion; in the former case the water currents move obliquely backward; in the latter case the animal moves on the same lines obliquely forward. The Paramecium is, as it were, whirled about in its own whirlpool" (*loc. cit.*; p. 305). An inspection of the Fig. 19 referred to in this extract shows that when the animal moves in a circle as there described, the oral side of the Paramecium must look continually toward the centre of the circle. Fig. 10 shows the successive positions of a Paramecium exhibiting the thigmotactic reaction toward such a movable particle; the arrows indicate the direction of motion of the Paramecium, while the circle indicates the path traced by the anterior end of the animal. By reference to this figure it will be noticed that under these circumstances the body moves forward tracing a curve, with the oral side always toward the inside of the curve; in other words, the animal continually turns *toward the oral side*. In connection with the other movements de-

scribed in these pages, it is important to recall the fact that Paramecia will be sometimes seen thus turning toward the oral side; this, however, is always in the thigmotactic reaction. It is especially frequent when Paramecia are observed undisturbed in water containing many bacteria; when the Paramecia are excited by some unusual condition this reaction is not seen. Now, it is chiefly the reaction under some exciting stimulus that forms the subject of this paper. The thigmotactic reaction is characteristic for resting individuals, and the locomotion in thigmotaxis under certain circumstances in the manner shown in Fig. 10 is an accidental matter, due to the mobility of the solid inducing the reaction. Hence, this is not properly to be accounted a motor reaction, and it is, therefore, with this brief description, left out of consideration in my discussion.

Returning, therefore, to the proper motor reactions, the final step must now be taken; the mechanism of the reactions already described must be determined in terms of the movements of the cilia. All movements of the Paramecia result from motions of the cilia, and the mechanism of the reactions cannot be considered as analyzed into its simplest components until we know what motions of the cilia are at the basis of the general body movements. For studying the motions of the cilia two methods may be used. The first is the observation of the animals in the gelatine solution in the way already given. The thickness of the solution makes the motion of the cilia much less rapid, so that the way in which they move can be determined more or less satisfactorily. The data gained in this way may be supplemented and controlled by the second method of study. This consists in observing the Paramecia in fluid containing powdered carmine. The movements of the particles of carmine in the neighborhood of the animals show the currents caused by the cilia, and from the direction of these currents the direction of stroke of the cilia causing them may be inferred.

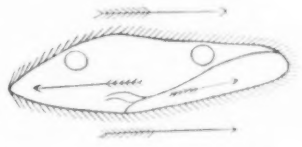

FIGURE 11. Paramecium with cilia reversed. The large arrow in the body shows the direction of locomotion of the animal; the other arrows indicate the direction of the currents of water.

In the unstimulated Paramecium swimming forward the effective stroke of all the cilia is backward, urging the animal forward; the cilia as seen in gelatine give the optical effect of being directed backward, as shown in Fig. 1. On coming in contact with an effective

source of stimulus, the cilia are reversed in position, now striking more strongly forward, and presenting the appearance of being directed toward the anterior end, as in Fig. 11. As a result the animal is, of course, impelled backward, or toward the broad, pointed end. It is worthy of especial notice that the cilia in the oral groove are, like all the others, now striking forward, so that a current of water passes swiftly *from* the mouth toward the anterior end, — contrary to the usual direction.

Next, after the backward swimming caused by the reversal of the ciliary motion has ceased, the animal begins to turn. At this stage the cilia are found to have the following motions. The reversal of the cilia in the oral groove has ceased, and they are striking backward again, — thus tending to drive the animal forward. The remainder of the cilia on the anterior half of the animal strike *transversely toward the oral side*. This is shown in Fig. 12. This reaction was first seen clearly in Paramecia that were immersed in a $\frac{2}{5}$ per cent solution of chrome alum; it was then confirmed for other substances and for stimuli other than chemical, by the use of the gelatine solution and by observation of the currents about Paramecia in water containing powdered carmine. The arrows in Fig. 12 indicate the direction of the water currents produced by the cilia at this stage of the reaction. In the oral groove there is a strong current backward, while on the anterior half of the aboral side there is a transverse current from the aboral to the oral side. This motion of the cilia of course causes the Paramecium to turn toward the aboral side (opposite the arrows in Fig. 12), and with this observation we have determined the real mechanism of the turning.

FIGURE 12. Position of the cilia as the Paramecium turns. The arrows indicate the direction of the water currents.

The next step in the reaction is the cessation of the transverse stroke of the aboral cilia, so that they strike backward, as before the stimulation, driving the animal straight forward as at first.

Thus the reaction of Paramecium to any ordinary stimulus is as follows: (1) The cilia over the entire surface of the body are reversed, striking forward, thus driving the animal backward. At the same time its direction of revolution on the long axis is reversed. (2) The reversal of the cilia ceases; those in the oral groove strike

backward as at first, while those on the remainder of the anterior half of the body strike transversely, toward the oral side. This results in turning the anterior end toward the aboral side. (3) The cilia all strike backward as at first; the animal, therefore, swims straight forward again. Coincidently with (1) and (2) the body of the animal may be bent toward the aboral side, still further aiding in turning in that direction. At the end of the entire manœuvre the Paramecium is swimming forward along a course which lies at an angle to the course in which it was first swimming. The complete reaction of Paramecium to an ordinary stimulus is represented in

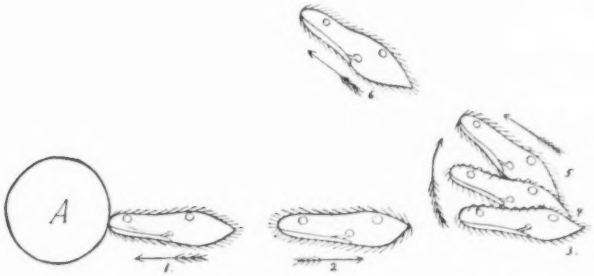

FIGURE 13. Complete reaction of a Paramecium. *A* is a source of stimulus. The numerals give the successive positions of the animal, while the arrows show the direction of motion.

Fig. 13. The rotation on the long axis could not be represented in this diagram, but all the other essential features of the reaction are shown.

This reaction is not characteristic for any particular class of stimuli, but it occurs in a more or less marked manner whenever any stimulus acts upon the animal with sufficient power to cause a motor reaction. Toward acids and alkalies, toward neutral salts and toward fluids that are active only through their high osmotic pressure; toward heat and cold and mechanical shock, the same reaction is given. Apparently a strong electric current has a related effect, since Verworn has observed that when a very strong electric current is passed through water containing Paramecia, they all swim backward, and thus collect at the anode instead of at the cathode, as is usual with a weaker current. A re-examination of the phenomena of electrotaxis in the light of the general reaction-plan described in this paper appears desirable to determine the relation of the two. Geotaxis, or

the reaction of Paramecium relative to the direction of the pull of gravity, in virtue of which they usually rise to the top of a vessel of water containing them, seems more difficult to bring into relation with the general reaction above described. In view of the divergent theories of Jensen,[1] Davenport,[2] and others, the cause of geotaxis cannot be considered certainly known; a reinvestigation of the phenomenon in Paramecium is needed, in the light of the general method of reaction described in this paper, as well as in view of the previously unsuspected positive chemotaxis toward carbon dioxide (as described in No. I of these Studies) — a substance the distribution of which in the water profoundly influences the movements of the Paramecia. Like Miss Platt,[3] I find that the Paramecia of this region do not show clear geotaxis.

Almost the only variation in this entire reaction is in the intensity of its several component activities. To a very weak stimulus, such as a mechanical obstruction in front, the Paramecium responds merely by a reversal of the cilia lasting but an instant and carrying the Paramecium backward but a fraction of its own length. The accompanying reversal of the direction of rotation on the long axis may suffice to turn the aboral side but a few degrees from its original position. The reversal is followed by a momentary transverse stroke of the aboral cilia, turning the Paramecium through but a small angle toward the aboral side. It then swims forward as before. The whole operation has caused a hardly perceptible stoppage in the course of the Paramecium. On the other hand, coming in contact with some strong chemical, such as a drop of $\frac{1}{100}$ per cent sulphuric acid, induces an intense and long sustained reaction. The Paramecium darts swiftly backward many times its own length, revolving rapidly on its long axis from right to left. Finally, the transverse stroke of the aboral cilia intervenes, in an equally powerful manner, and the animal turns sharply. It may thus turn completely around; or in some cases it may whirl more than 180°, so that the immediately following forward course takes it back again into the drop of acid. Certain chemicals exaggerate all the activities composing the reaction to a remarkable degree. Thus in $\frac{3}{4}$ per cent potassium iodide, as already described, the reversal of the cilia lasts six to eight minutes, the Paramecia all this time swimming swiftly backward; then the transverse

[1] JENSEN, P.: Archiv f. d. ges. Physiol., 1893, liii, p. 428.

[2] DAVENPORT, C. B.: Experimental morphology, Part I.

[3] PLATT, JULIA B.: American naturalist, 1899, xxxiii, p. 31.

stroke of the aboral cilia lasts perhaps half an hour, the animal all this time whirling toward the aboral side. In such cases the reaction loses all semblance of an attempt to escape or pass by an obstruction. In other chemicals each component part of the reaction is little exaggerated, but the entire operation is repeated many times, alternations of swimming backward, turning, and swimming forward again continuing for fifteen minutes or more. Different chemicals have a most varied effect on the reaction as a whole, though in every case the effect may be expressed as a modification of the intensity or duration of one or more of the component activities.

The absolute direction in which the Paramecium turns after its backward course is evidently determined by the position of the aboral side when the cilia on that side begin to strike transversely. The position of the aboral side at that time is a matter of chance; the animal revolves continually on its long axis, so that the position of the aboral side is continually changing, and the actual direction in which the Paramecium turns depends upon where the aboral side happens to lie when the reaction reaches the stage in which the aboral cilia begin to strike transversely. Of course this may carry the animal directly into the source of stimulus again; such cases are often observed, as has already been described and illustrated in Fig. 7. In many cases the Paramecium repeats the reaction several or many times before it succeeds in getting by the obstacle. The whole proceeding is an apotheosis of chance. If the source of stimulus is small, the process of drawing back and turning in any chance direction is likely to avoid it. Or, if it is not avoided the first time, the continual revolution on the long axis is likely to bring the aboral side into a new position next time, so that the Paramecium turns in a new direction. If this is continued long enough, a small obstruction is certain, by the laws of chance, to be evaded and passed after a sufficient number of trials. If, on the other hand, the source of stimulus is large; if, for example, the space containing the Paramecium is bounded along one whole side by a solution toward which the Paramecium is negatively chemotactic, then the reaction is repeated until by the laws of chance the course of the Paramecium is so directed as to lie in that part of the space which does not contain the repellent solution. In the case of a solution toward which the Paramecium is strongly negatively chemotactic, chance may be aided by the fact that the intensity of the stimulus usually causes an intense reaction, so that the Paramecia swim a considerable distance

backward (which in itself, of course, increases the chance of avoiding an obstacle after turning), and also turn strongly, — perhaps nearly 180°, so that the remainder of the path lies in quite a new direction. It seems probable that for most circumstances in the normal daily life of a Paramecium, this rough hit-or-miss method of reaction is quite sufficient to keep the animal out of danger and to enable it to avoid obstacles. But the utility of the reaction is left to chance, and so circumstances may arise in which the chances are against the Paramecium. Thus in the presence of a drop of some strong chemical, such as $\frac{1}{100}$ per cent sulphuric acid or cupric chloride, it will readily be seen that the chances of escape are limited. The animal responds to the first stimulus by a violent reversal of the cilia, carrying it some distance backward. If now the direction in which the transverse stroke of the aboral cilia carries it after this backward course is by chance such as to take the animal away from the drop, well and good; if, on the other hand, the Paramecium is carried a second time against the drop, it is likely to succumb at once to its poisonous effects. Observation shows that when a drop of such a substance is introduced into a preparation of Paramecia on the slide, it soon becomes surrounded by a zone of the dead infusoria. The same is true of a drop of a dense solution of cane sugar, which is injurious from its osmotic pressure. The Paramecia react when they enter such a dense solution, but the reaction is not precise enough to take them out of the destructive area before they are killed.[1]

The uselessness of the reaction seems especially striking when the Paramecia are completely immersed in a solution that acts as a stimulus. Under these circumstances, when there is no obstacle to be avoided and the stimulus is not localized at all, the Paramecia respond with exactly the same reaction. Paramecia do not modify their conduct to suit the needs of the case, but give the same reaction under all circumstances. The reaction is exactly such a one as might be maintained by natural selection, acting under an environment that makes no severe demands. Since the Paramecium usually swims forward, a majority of the stimuli that affect it at all will affect the anterior end. The whole reaction to *any* stimulus is, therefore, based upon this position of the source of stimulus, without regard to the conditions in a given particular case. For stimuli acting upon the side of the body, the reaction is, therefore, a very imperfect one, the

[1] There is great variation in the repellent power of different chemical compounds. This subject will be treated by the author in a separate paper.

comparative rarity of such stimuli having led to no development of a special mechanism to suit that case. A reaction such as would permit a direct turning away from a stimulus acting upon any side of the animal would evidently involve a much more complicated mechanism, and imply a higher condition psychologically than this simple one-reaction plan. But even this serves, if repeated a sufficient number of times, for avoiding sources of stimuli acting upon the sides of the animal, and under some conditions the result is produced in a fairly direct manner. In certain cases, when the stimulus causing the reaction is very weak, and the Paramecium comes against the source of stimulus very obliquely, so as to touch it only on one side, there appears to be an almost direct turning away, toward the side opposite the stimulus. This is to be observed particularly in the collections of Paramecia in a region containing carbon dioxide excreted by themselves. A long narrow area may thus be formed beneath the cover-glass (as shown in Fig. 14), in which the Paramecia swim back and forth; to the water outside this area they are weakly negatively chemotactic, so that when they swim against the boundaries of the area, the above-described reaction takes place, in a not very pronounced manner. A single Paramecium swimming lengthwise of this area may strike against its lateral boundaries with one side or the other, when it will frequently (not always) be seen to apparently sheer directly off, away from the boundary, and keep on its course undisturbed. Careful observation shows that such cases are to be placed in two categories. (1) When the Paramecium comes against the boundary, the aboral side by chance already lies toward the interior of the area, so that all that is necessary is for the Paramecium to react in the usual manner, in order to leave the boundary. (2) The more interesting case is where the Paramecium comes against the boundary with the aboral side *not* toward the interior of the area. In this case it will be seen to hesitate an appreciable interval before turning away from the boundary. During this moment of hesitation it *starts* to turn toward the aboral side, which is thereby pushed further into the outer fluid, toward which the animal is negative. This induces a faint reversal of the cilia (in agreement with the general

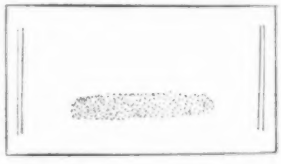

FIGURE 14. Group of Paramecia in a region containing carbon dioxide excreted by themselves.

plan of reaction above described), so that the animal does not progress further in that direction, and may be seen to move backward a barely perceptible distance. It then tries the turning again. On account of the continual rotation on the long axis, the aboral side will now be in a new position; the animal will, therefore, turn in a new direction, and may swim victoriously into the area; if not, a new trial is made during another instant of seeming hesitation. The delay cannot be longer than it requires for the Paramecium to rotate half a turn on its long axis. The animal makes the appearance of feeling about with its anterior end, to discover the proper course to pursue; it is really trying all the time to turn toward the aboral side, but cannot go forward until this carries it into a medium that does not cause a reversal of the cilia. The direction in which the animal shall go is not selected by the Paramecium itself, but is *automatically* selected through the fact that this direction does not cause a reversal of the cilia.

This more direct method is, however, only possible in cases where the negative stimulus is very weak, so as to cause only a very faint reversal of the cilia. With a stronger stimulus the animal shoots back as much as half its length, or more, so that it is now separated from the source of stimulus by a perceptible interval (as in Fig. 13); it is, therefore, free to turn in any direction. Hence, it as frequently turns at first *toward* the source of stimulus as in any other direction. Moreover, even with the weakest stimulus, not all individuals turn in the more direct way just described; many individuals in such an area as shown in Fig. 14 react essentially as shown in Fig. 13. The latter figure shows the *typical* reaction, of which the method just described is a less usual special case.

For the case of complete immersion in a solution that acts as a stimulus, the reaction seems under experimental conditions to be ludicrously imperfect and useless. Under the normal conditions, however, it perhaps serves the Paramecium very well even for this case. Suppose that the Paramecium in its headlong course finds that it has entered completely a circumscribed region of solution having injurious properties. Evidently, to immediately reverse the cilia and swim a long distance straight backward is as likely to rescue the Paramecium from its perilous position as anything it could do, and if this straight course backward does not bring it at once out of the injurious solution, it is perhaps likewise sound practice to change the direction of motion. As under other circumstances, the reaction

depends upon chance for success, but the chances are fairly favorable. It is interesting to see how so simple and invariable a method of reacting can meet so well the demands made upon it.

Finally, for the very rare case of stimuli acting upon the posterior end, no provision whatever seems made. It is only when swimming backward in response to a stimulus from in front that a stimulus is likely to act upon the posterior end, — and this is apparently so rare that it can safely be neglected. In cases where animals swimming backward come in contact with something that would act as a stimulus if it affected another part of the body, the cilia continue to strike forward. The normal reaction would be of course to reverse the stroke of the cilia, making them strike forward; as, however, they are already striking forward, there is now no change. Thus Paramecia swimming backward may often be seen to press the posterior end against a mechanical obstruction met in the backward course, and remain there, the forward stroke of the cilia driving them continually against it. Or in the case of chemical stimuli, Paramecia swimming backward often cross the boundary between two substances, when this boundary seems an insuperable obstruction to individuals swimming forward. This is particularly striking when a drop of weak acid is introduced into a slide of Paramecia. As detailed in my previous paper (*loc. cit.* p. 269, Figs. 7 and 8), the Paramecia gather in a ring about such a drop, and are then negatively chemotactic to the inner, more strongly acid part of the drop, and also to the outer fluid. Therefore, they swim about in a ring of some width, surrounding the drop of acid; if they strike against either the inner or the outer boundary of this ring, the cilia are reversed, so that the animals return into the ring. But if a Paramecium on striking the drop of acid at the inner boundary of the ring reverses its cilia strongly and swims backward, it may, if it reaches the outer boundary while swimming backward, cross this boundary without the slightest hesitation, and swim away into the surrounding water, though if it had come against the outer boundary while swimming forward, it would have been at once forced back into the ring by the reversal of its cilia. In cases, however, where a Paramecium swimming backward comes in contact with a source of very strong stimulus, the next step in the reaction is induced, — the Paramecium begins whirling on its short axis, — a proceeding which serves a purpose as little as would the continued swimming backward.

The collection of Paramecia in the region of a given attractive

stimulus, as in positive chemotaxis or positive thermotaxis, evidently depends equally upon chance. Nothing acts as a direct attraction to Paramecia. Their habit of swimming continually through the water is what must be relied upon to bring them into the sphere of activity of any agency. In this seemingly random swimming they are frequently met by influences which cause in a slight degree the reaction I have described, as is shown by the frequent slight jerks backward, followed by a slight turn in a new direction. In this way the Paramecium follows the line of least resistance, as it were, and finally may come into the region of a chemical toward which it is positively chemotactic, or into a region of optimum temperature. If now it proceeds on its course across the optimum territory, on coming to the farther boundary it passes into a region of such a nature as induces the negative reaction; the animal turns away and after some repetitions is directed back into the optimum region. The actual process by which this takes place in the case of thermotaxis may be detailed, since my account differs so completely in principle from that of Mendelssohn,[1] who first gave a careful description of thermotaxis, — and since this process affords a very striking example of what might be called the automatic selection of movements.

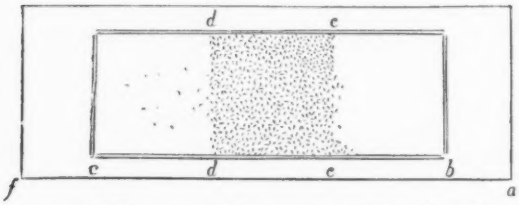

FIGURE 15. Thermotaxis of Paramecium. The right end from *a* to *c* rests upon a water bath heated to 40° C.; the opposite end, from *f* to *d*, rests upon ice.

Four strips of capillary glass tubing, enclosing a rectangular space, are fixed on a slide by means of Canada balsam (Fig. 15); the enclosure is then filled with water containing Paramecia, and covered with a long cover-glass. The Paramecia are scattered uniformly throughout the enclosure, swimming in all directions (the two ends being at first not empty, as they appear in the figure). Now one end of the slide for about a fourth of its length (from *a* to *c*) is allowed to rest on a water bath heated to about 40° C., while the other end, from *f* to *d*, rests on a piece of ice. Soon it will be noticed that the Paramecia at the heated end, between *b* and *c*, are in violent mo-

[1] MENDELSSOHN, M.: Archiv f. d. ges. Physiol., 1895, lx, p. 1.

tion. They swim rapidly, some forward, some backward, now and again turning quickly and shooting in a new direction. No particular direction is preferred; the animals simply swim swiftly in any chance direction. Now those which by chance cross the line *c—c*, coming into a region of low temperature to the left of *c*, cease their violent movements, while those which have started in a different direction keep up their headlong course until they also cross the line *c—c*, which they must inevitably do, purely by the laws of chance, if they continue to be active. Thus in time all the Paramecia which were in the heated region at the right of *c* will have crossed the line *c—c*, leaving the heated space empty. Meanwhile, it is not filled again, because any Paramecia to the left of *c* that may swim toward the heated end, react by reversal of the cilia and turning, when they come to the line *c—c* separating the hot from the cooler region. Thus the Paramecia have entirely vacated the region to the right of *c*, simply by keeping up their undirected movements, until by chance a practicable one carries them across the line. At a certain time after the beginning of the reaction a stream of Paramecia will be seen crossing the line *c—c* from right to left, since any other movement in this region causes a reversal of the cilia and turning.

At the other end, in the region resting on the ice, the cold does not act like the heat in greatly heightening the activities of the animals; it does, however, stop the thigmotactic reaction, so that all the Paramecia are set in motion, though perhaps very slowly. (If the cold is allowed to act suddenly and strongly, the Paramecia are simply benumbed, and remain where they are.) Some of the Paramecia thus swim by chance across the line *d—d*; the reaction is not, however, so quick and sharp as in the case of too high a temperature, and a number of stragglers will remain benumbed in the cold region. But those which are to the right of *d*, whether originally so, or after having crossed the line *d—d* from the cold region, do not cross this line again, since when they come to the line the cold causes a reversal of the cilia and a turning away. The cold region, therefore, gradually becomes emptied of Paramecia, like the hot space at the opposite end of the slide. The Paramecia in the middle region of the slide may now swim undisturbed anywhere between *c* and *d*, but as soon as they attempt to swim across the line *c—c* or *d—d*, the reaction sets in, and they are turned back. After a time, therefore, practically all the Paramecia are gathered in the optimum region between *d* and *c*, while the ends of the enclosure are empty. The

result is not due to direct swimming toward the optimum temperature, nor a direct swimming away from the hot or cold region, but by a sort of "automatic selection" of movements. The Paramecia in the two ends keep swimming hither and thither, until a chance movement in the right direction carries them into the optimum zone. Motion takes place in any direction till one is automatically selected by the fact that it brings the Paramecium into new conditions. The analogy of this to the natural selection of structures is obvious.

Mendelssohn, who described the phenomena of thermotaxis in Paramecium without a knowledge of the finer mechanism of the reaction, assumed that the Paramecia turned and swam *directly* toward the optimum region from either end. In a Paramecium approaching a source of heat, the anterior end would thus be slightly warmer than the posterior end. Mendelssohn assumed that the directive control exercised by temperature must be due to this slight difference in temperature between the anterior and posterior ends of the same animal. He found that in a trough 10 cm. long, a difference of three degrees Celsius between the two ends of the trough was sufficient to cause the Paramecia to collect in the warmer end. The average length of a Paramecium being about 0.2 to 0.25 mm., it was easy to calculate that there was a difference in temperature of about 0.01° C. between the two ends of a Paramecium swimming lengthwise in the trough. It was, therefore, concluded that the Paramecia were sensitive to a difference in temperature of 0.01° C. But, as shown above, the method of reaction is much coarser than that assumed by Mendelssohn; the animal swims from one side of the optimum region to the other, not reacting at all until it comes to a region decidedly hotter or colder. The sensitiveness to temperature differences may, therefore, be much less than that given by Mendelssohn. In a trough with the optimum temperature at one end and changing gradually to the other, the only difference that one can be certain of their appreciating is that between the positive and negative limits of their excursions. If a single Paramecium swims back and forth over a stretch of 5 mm. lying between the cooler and warmer regions of the trough, then it must be concluded that it is sensitive to the difference in temperature between points 5 mm. apart; sensitiveness to any less difference is not proved. Similar considerations apply to other sources of stimuli, since the mechanism of reaction is the same for all classes of stimuli. It is evident that the sensitiveness of Paramecium to different agents may have been greatly overestimated.

Moreover, similar deductions as to the sensitiveness of other organisms must be considered insecure until the exact mechanism of their reactions has been determined.

While the general reaction I have described is evidently from certain standpoints an extremely simple one, yet it bears strongly marked the distinctive features of reactions to stimuli as we know them in the higher organisms, — namely, the lack of any direct and apparent relation between the effect and the cause. In studying the activities of inorganic matter, it is possible to establish relationships of cause and effect — often through a somewhat extended chain from the first cause to the final effect — that may be clearly perceived as causal relations, — so that the effect could be predicted from a knowledge of the first cause and the attendant conditions. On the other hand, in dealing with the activities of living bodies, we are confronted with phenomena in which the steps connecting cause and effect cannot be traced; such phenomena are called reactions to stimuli. Evidently, one of the chief objects in the investigation of the activities of living bodies must be the attempt to resolve reactions to stimuli into the chain of causes of which we must believe them to be composed, so that a series of steps can be traced, each satisfactory from a causal standpoint, from the original cause to the final effect. Can we hope by a study of the simplest unicellular organisms to approach such a resolution of life activities into connected chains of cause and effect? As a hypothetical example of such a resolution may be cited Verworn's theory of the chemotactic movements of Amœba toward oxygen; if this theory is true, the relations of cause and effect are clearly traced from the original cause to the final effect. In the case of the comparatively complicated organism Paramecium, it was perhaps scarcely to be expected that any clear evidence of the actual causal relation between stimulus and reaction should be made apparent, and as a matter of fact, we find, from a causal standpoint, an absolute gulf between them. It is not even possible to trace such general relations between the contractility of the cilia and the nature and position of the sources of stimuli as have been assumed by various authors; Jensen,[1] for example, assumes that a stimulus acting upon one side of the Paramecium causes the cilia of that side to beat more strongly, thus turning the animal away from the source of stimulus; some such relation might perhaps have been reasonably anticipated. But we find that the most varied and

[1] Jensen, P.: Archiv f. d. ges. Physiol., 1893, liii, p. 471.

even opposite stimuli produce the same reaction, and that stimuli at one end, at the side, or acting over the entire surface of the animal at once, produce the same result. The reaction appears to be purely arbitrary, without any relation to the nature of the cause. It evidently depends upon the internal mechanism of the body of Paramecium, and this mechanism is so complicated that we are quite unable to trace the steps which lead from cause to effect.

For all practical purposes, therefore, the reactions of Paramecia present phenomena not a whit simpler for a causal understanding than the activities of a highly developed Metazoan. The mechanism of the unicellular organism, lacking as it does a complex nervous system, may, as a matter of fact, be simpler than that of a Metazoan, but in both cases the mechanism is of sufficient complication to interpose for our understanding an absolute break between stimulus and reaction; hence the study of the Protozoan gives no deeper insight into the essential nature of such reactions than does the study of a Metazoan. Whether the reactions of such a comparatively undifferentiated lump of protoplasm as Amœba will really prove of any more value for such an insight remains to be seen; certainly Amœba is deserving of a thorough experimental study from this standpoint.

Thus the principal intrinsic interest of this investigation lies not so much in the fact that the organism studied is a mass of protoplasm constituting but a single cell, as in the fact that it represents a very low place in the psychological scale. From the psychological standpoint, the reactions of Paramecium are extraordinarily simple. We have in this animal perhaps as near an approach to the theoretical reaction postulated by Spencer and Bain for a primitive organism, — namely, random movements in response to any stimulus, — as is likely to be found in any living organism. The motions are strictly random, so far as the position of the source of stimulus is concerned; the animal always swimming, after stimulation, in the direction of one of its own ends (the posterior), and turning toward one of its own sides (the aboral), without regard to the relation of these directions to the source of stimulus. And by the repetition of the reaction the direction of movement is frequently changed, — always without relation to the localization of the stimulus. It appears not to have been foreseen, theoretically, that such random movements would of themselves if continued carry the animal out of the sphere of influence of the agent causing them and keep it from re-entering. To accom-

plish this result, it is only necessary that the direction of motion should be changed at the moment when the stimulus begins to act, and at intervals so long as its action continues.

It is evident from this method of reaction that Paramecia are neither directly attracted nor directly repelled by any agencies. It is often assumed that it is a universal property of living organisms to react in two opposite ways toward stimulations, — to be attracted by some and repelled by others. In view of the reactions of Paramecium this assumption must fall to the ground, as certainly not universal, and possibly not nearly so general as has been supposed. The mechanism of the reactions of most simple organisms remains yet to be ascertained.

In regard to the position in the psychological scale to be assigned to Paramecium, the following may be said. The organism responds to any stimulus by a definite, well-characterized reaction. The same may be said of the isolated muscle of a frog. The intensity of the reaction varies with the nature and intensity of the stimulus; this also is true for the muscle. Under certain influences the Paramecium remains quiet; likewise the muscle. The *directive* relations of the motions performed are determined both in the Paramecium and in the muscle by the structure of the organism, not by the localization of the stimulus. There seems, then, no necessity for assuming anything more in order to explain the reactions of Paramecium than to explain the reactions of the muscle. We require, therefore, little or nothing more than irritability, or the power of responding to a stimulus by a definite movement, to account for the activities of Paramecium. Since the direction of motion of the Paramecium has no relation to the position of the source of stimulus, there is no need to suppose that the animal has anything related to a *knowledge* of this position. Moreover, it exercises no *choice*, — the direction of motion being always the same with reference to the parts of the animal. I do not see that we are compelled to assume consciousness or intelligence in any form to explain the movements of this creature.

Summary.

1. Paramecium has a single motor reaction, by which it responds to all classes of stimuli. This reaction consists of the following activities. (1) The direction of the stroke of the cilia is reversed, over the entire surface of the animal, so that they strike forward, driving the animal backward. (2) The reversal ceases; the cilia in the oral

groove strike backward, as at first, while the aboral cilia of the anterior half of the body strike *transversely toward the oral side. This results in turning the animal toward the aboral side.* The anterior end may, if the stimulus is strong, be curved toward the aboral side, thus aiding in turning toward that side. (3) The cilia all strike backward as at first, driving the animal forward again. At the end of the reaction the Paramecium is swimming forward on a path which lies at an angle to the path on which it was moving at first.

2. The intensity and duration of the different parts of this reaction vary with the nature and intensity of the stimulus.

3. Paramecia do not turn directly toward a beneficial source of stimulus, nor directly away from an injurious source of stimulus, but the reaction is always as stated under 1. That is, localized stimuli, acting on one side or one end of the animal, have the same effect as stimuli acting upon the entire surface of the body at once. The direction in which the Paramecium turns after a stimulus is determined by internal factors, and is expressible in terms of the animal's structure; it has no relation to the position of the source of stimulus. The *absolute* direction in which the animal turns is a matter of chance; if it does not carry the animal away from the obstruction the first time, the entire reaction is repeated till it does.

4. *Positive* and *negative taxis*, as applied to Paramecia, are merely convenient terms for expressing the fact that the animals form collections in the regions of certain influences and do not form such collections in others; the terms do not express motor reactions. Paramecia are not directly attracted or repelled by any agencies or conditions. The collections formed in the regions of certain agencies are due to the fact that these agencies cause *no motor reaction.*

5. Different classes of chemical or physical stimuli do not have qualitatively different methods of affecting the stroke of the cilia.

6. In view of the mechanism of the reactions, the sensibility of Paramecium to different agents has probably been much overestimated, since it is not the differential effect on the anterior and posterior ends of the animal that induces the reaction.

7. In the reactions of Paramecium the steps between cause and effect cannot be traced, opposite causes producing the same effect, and the same cause if continued producing opposite effects. The nature of the reaction is conditioned by the internal mechanism of the animal's body.

8. The reactions of Paramecium indicate that the organism stands at the very bottom of the psychological scale. Simple irritability, or the property of responding to a stimulus by a fixed set of movements, seems sufficient to account for its activities.

The results presented in the foregoing paper were obtained in connection with an extended study of the chemotaxis of Paramecium, carried out in the Laboratory of the United States Fish Commission for the Biological Survey of the Great Lakes during the summer of 1898, and continued at Dartmouth College during the following autumn. It is a pleasure to acknowledge here my indebtedness to Prof. J. E. Reighard, the Director of the Survey, and to the officials of the United States Fish Commission for assistance and courtesy in every way in carrying on this investigation, as well as for permission to publish the present paper.

Dartmouth College,
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ON ABSORPTION FROM THE PERITONEAL CAVITY.

By LAFAYETTE B. MENDEL.

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THERE is abundant experimental evidence to indicate that when an intravascular fluid is separated from an extravascular one of different composition, an interchange of substances will take place between them. Given a separating membrane, *e. g.* a capillary wall, sufficiently permeable to the substances in solution, the two fluids will manifest a tendency to become equalized in composition. Thus substances originally dissolved in the extravascular fluid only may pass into the intravascular solution according to the established principles of osmosis.[1] That substances foreign to the blood can in this way be absorbed into the circulation directly through the blood vessels has long been known.[2] In investigating the absorption of dissolved substances with reference to the channels by which they enter the circulation from the connective tissue spaces, it has been a customary procedure to introduce readily detectable compounds into these spaces, and to watch for their appearance in the blood or lymph stream under various conditions. The outcome of these researches has been to attribute to the blood vessels an important rôle in absorption from connective tissue spaces, quite in accord with the requirements of physical diffusion; although it has by no means been denied that the lymphatics also may be engaged in the transfer from the tissue cavities to the blood.

The problem of absorption from the pleural and peritoneal cavities has been studied by Starling and Tubby[3] among others. They introduced a colored substance — indigo-carmine or methylene blue — in solution into the cavities, and observed the reappearance of the pigment in the urine after ligation of the thoracic ducts, or after introducing cannulas and collecting the lymph before its entrance into the blood stream. In their experiments the urine was collected

[1] Cf. Róth: Archiv für Physiologie, 1898, p. 542.

[2] Cf. Schaefer's Text-book of Physiology, 1898, i, p. 303; Munk, I.: Archiv für Physiologie, 1895, p. 387.

[3] Starling and Tubby: Journal of physiology, 1894, xvi, p. 140.

through a cannula tied into the ureter, "or in most cases into the bladder, either through an opening into the abdominal wall, or through an opening in the urethra, which was exposed by splitting the symphysis pubis." They observed in this way that "5–20 minutes after the injection of the coloured fluid into the serous cavities the urine became tinged with blue, when indigo-carmine was used, or green, when methylene blue was the substance employed. The urine, on shaking with air, rapidly deepened in tint, until the colour was as intense as that of the fluid injected. After a further lapse of time, varying from ten minutes to four hours, the lymph flowing from the thoracic duct also became slightly tinged, but the colour never deepened, on shaking with air, beyond a very light blue or green. The flow of lymph was not increased" (p. 143). The investigators add: "It might be argued from these experiments that the absorption of the colouring matter from the pleural or peritoneal cavity took place by means of the blood vessels and by means of the lymphatics, the former process however being the quicker of the two. It is very doubtful however whether the slight colouration of the lymph which was observed in these experiments is occasioned at all by lymphatic absorption. If methylene blue or indigo-carmine be injected into the blood stream, the lymph flowing from the thoracic duct within half a minute becomes coloured. Now in these cases the colouring matter must have been present in the blood in order to have been excreted by the kidneys, and the colour of the lymph may be caused by a passage of coloured lymph from the blood vessels, not by any direct absorption of the blue from the serous cavities" (p. 144). The data presented thus indicate a direct interchange between the fluid in the cavities and the blood in the vessels. Cohnstein,[1] likewise, has found these phenomena in direct accord with the principles of osmosis.

Comparable with the preceding observations, and indicating the immediate importance of the blood vessels in absorption within the tissues, are experiments by I. Munk.[2] He severed the truncus lymphaticus colli, which conducts away the total lymph from the head in the rabbit, and removed the lymph coming from the cephalic end of the duct. When strychnine was thereupon injected under the skin of the head, no delay in the oncoming or intensity of tetanic symptoms was observed, although the blood vessels formed the only

[1] Cohnstein: Centralblatt fur Physiologie, 1895, ix, p. 403.

[2] Munk, I.: Archiv fur Physiologie, 1895, p. 387.

channel of communication with the general circulation. Furthermore, no strychnine could be detected in the lymph collected from the head. Again, Wertheimer and Lepage[1] have demonstrated the active participation of the blood vessels of the liver in the absorption of substances from the bile. When a solution of indigo-carmine was allowed to flow into the ductus choledochus of a dog at a pressure of 30 cm., the urine collected from the ureter was found to be blue some minutes before the lymph flowing from the thoracic duct showed a trace of the pigment. The authors conclude that the lymphatics play a subordinate part, at most, in this process of absorption.[2]

In a recent paper Meltzer[3] has reviewed the investigations of Starling and Tubby. Admitting that their experiments "if confirmed, would indeed prove the correctness of the blood vessel theory of absorption," Meltzer has repeated them and has presented the protocols of a series of further experiments which lead him to quite different conclusions. The method of experimentation was essentially that employed by Starling and Tubby. Colored substances, or potassium ferrocyanide, were introduced into the peritoneal cavity; the lymph was collected from the thoracic duct and the urine by means of a catheter introduced into the bladder through an external opening in the urethra. An extensive opening of the abdominal cavity was thus avoided. "In all of the experiments, the coloured fluid or characteristic Prussian blue appeared invariably in the lymph distinctly earlier than in the urine. . . . The lapse of time between the injection into the abdomen and the appearance in the lymph was in all the experiments approximately the same — an average of fifteen minutes. . . . On the other hand the time for the first appearance of the colour, etc., in the urine varied considerably in the different experiments." Meltzer adds: "In the few experiments I made by injecting coloured fluid directly into the circulation, the colour appeared in the lymph before it appeared in the urine; the interval for the lymph was approximately constant, while the interval for the urine was quite variable — between seven and thirty-four minutes."[4] In view of this reverse of

[1] WERTHEIMER and LEPAGE: Archives de physiologie, 1897, p. 373.

[2] Page 374: "Ces quelques exemples suffisent pour prouver non seulement que les vaisseaux sanguins prennent une part active à la résorption du pigment bleu, mais encore que les lymphatiques n'y ont qu'une part très restreinte."

[3] MELTZER: Journal of physiology, 1897, xxii, p. 198.

[4] MELTZER: *Ibid.* p. 203.

the result obtained by Starling and Tubby, Meltzer has rejected the theory that the peritoneal absorption takes place through the walls of the blood vessels and not by way of the lymphatics.

The preceding discussion has led Starling[1] to repeat his earlier experiments with due regard to the precautions emphasized by Meltzer, and he has failed to discover any fallacy in them. He calls attention, however, to the necessity of emptying the bladder at regular intervals of two or three minutes by pressure on the abdominal wall, when the urine is collected through a cannula inserted directly into the bladder. Otherwise the urine is apt to accumulate in the latter and only the overflow is collected; a delay is thus occasioned between the secretion of a portion of urine and its appearance in the cannula. To this point I shall refer again somewhat later.

In the course of some experiments on the action of certain lymphagogues[2] it occurred to me that if colored substances introduced into the abdominal cavity are carried into the circulation mainly through the lymphatics, an acceleration of the lymph flow might be accompanied by a more rapid or extensive transference of the pigment along this channel.[3] The first experiment was on a dog in which an intravenous injection of heteroalbumose had produced pronounced lymphagogic effects together with complete stoppage of urinary flow. Ten c.c. of strong indigo-carmine solution were introduced into the peritoneal cavity. No blue could be detected in the lymph collected within an hour; the bluish tint of a sample of blood serum, however, gave evidence of an active absorption. In another animal previously receiving an intravenous injection of hemipeptone, the influence of which had already disappeared, fifteen c.c. of the blue solution were introduced into the peritoneal cavity. The blue color appeared in the urine in *fourteen* minutes, in the lymph three minutes later. Observations of this kind led to a series of experiments on normal dogs under a variety of conditions. The technique of the experiments was essentially like that adopted by Starling and by Meltzer. The dogs were anæsthetized (after 36 hours' fasting) by a subcutaneous injection of morphine sulphate followed by chloroform-ether administration. Cannulas were tied into

[1] STARLING: Journal of physiology, 1898, xxii, p. xxii.

[2] CHITTENDEN, MENDEL, and HENDERSON: This journal, 1899, ii, p. 162.

[3] In this connection it was, of course, remembered that the lymph formed through the influence of these "lymphagogues" has its *origin* primarily in the liver.

the thoracic duct and both ureters. For collecting the urine the ureter method seems to me to be the preferable one, since it prevents any stagnation of fluid in the bladder. But inasmuch as the method has been criticised because it occasions the opening of the abdominal cavity the urine was collected in some cases directly from the bladder, in bitches, by introducing a catheter exteriorly through the urethra. The results obtained were not modified by this procedure, as the protocols will show. The colored fluid (indigo-carmine solution) was always introduced into the peritoneal cavity with a blunt pipette[1] through a small opening in the linea alba near the sternum. The abdominal wounds were all carefully stitched afterwards. The details of some experiments follow.

I. Dog, 16 kilos. Cannula in thoracic duct and in each ureter. The lymph flow in ten minutes was 2.3 c.c.; urinary flow was good. At 12.30, 20 c.c. strong indigo-carmine solution were introduced into the peritoneal cavity. At 12.46 the urine was tinged blue; the lymph continued normal in color until 12.58-12.59, when a faint blue tinge was evident. The urine, at this time, was perfectly dark with blue. At 1.05 the lymph showed a deeper blue, but the color was not intense; at 1.20 the color began to deepen, and still persisted when the experiment was concluded at 2.30. The urine continued to show a very dark blue color.

Thus the color appeared in the urine in *sixteen* minutes; in the lymph in *twenty-eight* minutes.

II. Dog, 20 kilos. Cannula in thoracic duct and in each ureter; cannula for bleeding in femoral artery. The urine and lymph flowed regularly, the latter at the rate of 1.9 c.c. in ten minutes. At 12.22 20 c.c. saturated indigo-carmine solution were introduced into the peritoneal cavity. A bluish tint appeared in the urine at 12.31, becoming very deep at 12.34. The color began to show in the lymph at 12.45 and gradually increased in intensity. At 12.47 a blood sample was drawn; the serum was obtained by centrifugalization and was precipitated with alcohol. The alcoholic filtrate was decidedly blue at a time when the lymph was only beginning to show a trace of color. At 2.30, when the experiment was concluded, the urine was still intensely blue with pigment, while the lymph was light blue.

Thus the color appeared in the urine in *nine* minutes; in the lymph in *twenty-three* minutes.

III. Bitch, 9 kilos. Cannula in thoracic duct. Catheter introduced exteriorly through urethra into the bladder. Lymph flow in ten minutes was

[1] ADLER and MELTZER caution against the use of a sharp cannula for this purpose, since it may enter the intestinal lumen occasionally: Journal of experimental medicine, 1896, i, p. 493.

1.9 c.c. The urine flowed readily whenever pressure was applied over the bladder; otherwise the flow was not regular, a few drops being expelled occasionally with respiratory movements. During the experiment the urine was expressed at intervals of about three minutes. At 4.01 15 c.c. strong indigo-carmine solution were introduced into the peritoneal cavity. At 4.10 the urine was distinctly blue, and the color had become intense at 4.20, when the first bluish tint appeared in the lymph. The color deepened somewhat, but had decreased, if anything, at the conclusion of the experiment at 5.05. A post-mortem examination showed the blue color well distributed throughout the peritoneal cavity.

In this experiment the possibility of escape of color along wounds in the abdomen was excluded. Nevertheless, the color appeared in the urine in *nine* minutes; in the lymph in *nineteen* minutes.

IV. Bitch, 10 kilos. Cannula in thoracic duct; catheter in bladder as in Exp. III. Urine was readily obtained by gentle pressure. The lymph flow in ten minutes was 2 to 3 c.c. At 11.35 2 c.c. indigo-carmine solution were introduced into the peritoneal cavity. At 11.53 the urine showed the blue color, which soon deepened. No trace of blue could be detected in the lymph within an hour. Accordingly at 12.43, 10 c.c. aqueous indigo-carmine solution were introduced in the peritoneal cavity. At 1.0 the urine was decidedly more blue than before; the lymph assumed a bluish tint (?) which became distinct at 1.05, growing deeper and remaining blue when the animal was bled to death at 2.15. Practically all the colored fluid had disappeared from the peritoneal cavity. The diaphragm alone was stained deep blue.

Thus the color appeared in the urine in *eighteen* minutes, while no blue could be detected in the lymph within an hour; and again in the urine *within seventeen* minutes, and in the lymph somewhat later. The experiment is interesting because of the method employed to collect the urine and of the *small amount* of indigo-carmine at first introduced.

The following details are included from additional experiments in which the lymph flow was accelerated by intravenous injection of a lymphagogue previously to the introduction of the colored solution into the peritoneal cavity.

V. Dog, 20 kilos. Cannula in thoracic duct and in each ureter. Cannula for intravenous injection in facial vein. The flow of urine and lymph being found satisfactory, at 11.33–11.34 50 c.c. of 20 per cent sodium chloride solution were infused intravenously. In the succeeding twenty-five minutes 46 c.c. of lymph and 112 c.c. of urine were collected. At 11.44 12 c.c. strong indigo-carmine solution were introduced into the peritoneal cavity. Blue color appeared in the urine at 11.48, and rapidly increased in intensity. At 11.53 the lymph assumed a faint bluish tint, which grew more pronounced, but was

never very deep. When the lymph flow diminished again, a solution of five grams of Grübler's "pepton" was infused intravenously at 12.15. The urinary flow ceased at once. The lymph continued blue in color, 26 c.c. being collected in the succeeding fifteen minutes.

Thus the color first appeared in the urine in *four* minutes; in the lymph in *nine* minutes.

VI. This experiment has already been referred to briefly. Dog, 15 kilos. Cannula in thoracic duct and in each ureter. A previous injection of heteroalbumose had stopped the urinary flow. The lymph flow in ten minutes was 4 c.c. At 5.0 10 c.c. indigo-carmine solution (in 0.7 per cent sodium chloride solution) were introduced into the peritoneal cavity. At 6.0 the serum obtained from a sample of blood showed a faint bluish tinge. No pigment could be detected in the lymph, which was still flowing steadily.

VII. Dog, 15.5 kilos. Cannula in thoracic duct and in each ureter. Cannula for intravenous injection in facial vein. After a solution of eight grams of Grübler's "pepton" had been introduced into the latter, the urinary flow practically ceased. The lymph flow in ten minutes was 10.6 c.c. Immediately after the infusion of the albumose-peptone 10 c.c. saturated aqueous indigo-carmine solution were introduced into the peritoneal cavity. The lymph continued to flow abundantly; no blue could be detected in it within three hours. A post-mortem examination showed the pigment distributed throughout the abdomen; the thoracic cavity was entirely free from it.

A similar experiment was carried out with analogous results on a dog of 20 kilos. Nearly 50 c.c. of lymph were collected without the appearance of any pigment in it being noted.

The data presented agree closely with those already recorded by Starling. In every case the characteristic color appeared in the urine before it could be detected in the lymph flowing from the thoracic duct, the interval observed ordinarily being about ten or twelve minutes. The reverse results obtained by Meltzer with indigo-carmine solutions are briefly tabulated below for comparison.[1]

Indigo-carmine Experiments of Meltzer.

Weight of Dog.	Amount of fluid introduced.	Time of first appearance of pigment in	
		Lymph.	Urine.
kilos.	c.c.	min.	min.
28	50	8	26
..	500	11	23
18	50	12	40
10	50	13	23
30	50	13	44
22	76	14	80

[1] MELTZER: Journal of physiology, 1897, xxii, p. 198.

In view of the criticisms of previous investigators, it may be well to point out that the tardy appearance of the pigment in the lymph has not been due to any retarded lymph flow in my experiments; the rate of flow varied ordinarily between 1.9 c.c. to 4.4 c.c. in ten minutes. The results are apparently independent of the size of the animal, and the data obtained by collecting the urine from the bladder agree in every respect with those furnished by the ureter method. The amount of colored solution introduced into the peritoneal cavity in my experiments has been relatively small compared with that used by Meltzer; this precaution has seemed desirable in view of the statement that "the absorption of such small amounts certainly afforded a greater similarity with that of the normal lymph than when the large quantities of fluids used by these other writers were employed."[1] Thus, in their experiments on rabbits, Adler and Meltzer used 1 to 2 c.c. of fluid.

It is not difficult to understand the prolonged absence of pigment from the lymph in those cases where only small quantities are introduced into the peritoneal cavity. The foreign substances, being quickly transferred directly into the blood stream, are at once taken up and eliminated by the kidneys, — as the intravascular injection of pigment also indicates. In this way little material is left to pass into the lymphatic system from the lymph spaces by this relatively slower process, or by subsequent transference from the blood vessels.

It has already been mentioned that Starling and Tubby express doubt regarding the direct absorption of coloring matter into the lymphatics in their experiments, and emphasize the possibility that the coloration of the lymph may be due to a "passage of coloured lymph from the blood vessels." Thus they observed that a colored substance introduced intravenously may appear in the lymph flowing from the thoracic duct within half a minute. So far as I am aware, no other comparable observations are recorded showing so brief an interval. For sodium salicylate, which does not influence the lymph flow, Tschirwinsky[2] has observed from four to seven minutes; Cohnstein[3] has found four or five minutes to intervene after potassium or sodium ferrocyanide solutions are injected, and for sodium iodide the writer[4] has repeatedly observed similar inter-

[1] Adler and Meltzer: Journal of experimental medicine, 1896, i. p. 484.
[2] Tschirwinsky: Centralblatt für Physiologie, 1895, ix, p. 49.
[3] Cohnstein: Archiv f. d. ges. Physiol., 1895, lix. p. 509.
[4] Mendel: Journal of physiology, 1896, xix. p. 227.

vals. A few additional data obtained with indigo-carmine are given here: —

VIII. Dog, 20 kilos. Cannula in thoracic duct and in each ureter; cannula for intravenous injection in right facial vein. Rate of flow of lymph in ten minutes was 2.3 c.c.; of urine was 1.1 c.c. At 11.59 to 12.0 10 c.c. of strong indigo-carmine solution (in 0.7 per cent sodium chloride solution) were infused into the vein. At 12.9 the blue color was distinct in the urine. At 2.45 there was still a good flow of urine and lymph; the latter had not yet shown a trace of blue, while the color of the urine had gradually grown less deep, now showing a light green tint. Therefore at 2.45 a further 20 c.c. of the same indigo-carmine solution were infused into the vein. At 2.55 the urine grew deep blue a second time, and at 3.08 the lymph began to show a faint blue tinge which scarcely increased in color. Lymph flow in succeeding forty minutes was 10 c.c. The dog was killed by bleeding and the thoracic duct showed no blue color in any part of its course.

Thus the color appeared in the urine in *ten* minutes; in the lymph in *twenty-three* minutes.

IX. Dog, 11 kilos. Cannula in thoracic duct and in each ureter; cannula for intravenous injection in right facial vein. The dog had been without food or water for two days; 50 c.c. 0.7 per cent sodium chloride solution had been infused slowly into the facial vein. Lymph flow in ten minutes was 3.1 c.c.; urinary flow was 0.9 c.c. At 11.42–11.44 15 c.c. strong indigo-carmine solution were slowly introduced intravenously. At 11.59 the urine was colored blue and quickly grew intense in tint. At 12.05 the lymph showed a faint bluish tint, which grew slightly deeper; but at 12.25 the color was doubtful. Therefore 25 c.c. indigo-carmine solution were again injected, whereupon at 12.33 the lymph again showed the blue tint, which deepened somewhat to light blue.

Thus the color appeared in the urine in *seventeen* minutes; in the lymph in *twenty-three* minutes (or with a larger quantity of colored solution in *eight* minutes.)

X. Dog, 15 kilos. Cannula in thoracic duct and in each ureter; cannula for intravenous infusion in left facial vein. Urine and lymph flow well; lymph in ten minutes was 3 c.c. At 12.20–12.21½ 20 c.c. one per cent indigo-carmine solution in one per cent sodium chloride solution were introduced into the vein. At 12.28½ the urine was deep blue; at 12.32 a trace of blue appeared in the lymph and gradually increased somewhat in depth of color. At 1.0 20 c.c. twenty per cent sodium chloride solution tinged with indigo-carmine were infused. The acceleration in the flow of urine and lymph immediately followed. The urine grew less deeply blue; the color was missed entirely in the lymph.

Thus the color appeared in the urine in *eight* minutes; in the lymph in *twelve* minutes.

In similar experiments Meltzer[1] has reported that the color appeared in the lymph before it appeared in the urine. Detailed protocols of the experiments have not been published, and few definite statements are made with regard to the urinary flow in these observations, or in those already tabulated. It is difficult to resist the conclusion that the differences between our results are perhaps attributable to variations in the method of collecting the urine. Thus in Meltzer's experiments on direct intravenous injection of colored fluid, intervals of *seven* to *thirty-four* minutes elapsed before color was detected in the urine; with intraperitoneal injections intervals of *twenty-three* to *eighty* minutes are recorded. The results were apparently independent of the size of the animal used. The longest interval that I have observed was *eighteen* minutes in Exp. IV when only 2 c.c. indigo-carmine solution were introduced. Furthermore the actual amount of pigment appearing in the lymph was in no case equivalent to more than a very small fraction of the pigment introduced into the animal, or found in the urine. A more satisfactory explanation does not suggest itself at present; there are, however, quite different and perhaps more satisfactory ways of arriving at an answer to the main problem concerned.[2]

The experiments recorded in this paper are thus in no way opposed to the "blood vessel theory" of the absorption of foreign substances from the peritoneal cavity. Rather they conform with the results which are demanded by the conditions and principles discussed at the outset.

[1] MELTZER: Journal of physiology, 1897, xxii, p. 203.

[2] In a recent paper on the absorption of various intraperitoneal fluids when the lymph channels are excluded (in the rabbit), Róth concludes: "Dabei findet eine langsame Resorption der isotonischen Lösung durch die *Blutgefässe* statt." Archiv für Physiologie, 1898, p. 545. The older similar experiments by Hamburger have been criticised by ADLER and MELTZER: Journal of experimental medicine, 1896, i, p. 482.

THE ORIGIN OF THE "TRAUBE" WAVES.

By HORATIO C. WOOD, Jr.

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RHYTHMICAL variations of blood pressure not associated with any perceptible alteration in the cardiac or respiratory function have been described by several observers. These waves differ more or less in their form and in the circumstances under which they are found. Traube,[1] who was the first to investigate the subject, mentions two types occurring in dogs in whom the artificial respiration had been suspended; the one is distinguished by a rhythm about that of natural respiration and resembles closely the respiratory variations in blood pressure; the other is much larger and slower. Mayer[2] observed waves of the latter class in rabbits either breathing naturally or supplied with artificial respiration. They appeared singly, at irregular intervals, or in rhythmical groups. Their cause was not apparent.

Since all forms of these waves are altered in their character in the same manner by similar influences the conclusion seems probable that the same cause underlies them all. When we come to look for this cause we find in the first place that the waves are due to changes in the vasomotor centre. The evidence in support of this is so universally acknowledged that I will mention only the experiment of Mayer, in which the temporary loss of function of the medulla produced by shutting off the blood supply causes the waves to disappear. The question as to what impels the vasomotor centre to send out these rhythmical impulses is not so easily answered. Hering[3] suggested that impulses pass over from the respiratory centre (irradiation). He grounds his belief on the correspondence in rate between the Traube waves and the respiration, and on the fact that sometimes in imperfectly curarized animals slight spasmodic movements of the diaphragm indicating activity of the respiratory centre,

[1] Traube: Centralblatt für die medicinische Wissenschaften, 1865, p. 881.

[2] Mayer: Sitz.-Ber. d. Kaiserl. Akad. d. Wissensch. zu Wien, Math.-nat. Kl., 3, 1876, lxxiv, p. 281.

[3] Hering: *Ibid.*, 1870, lx, p. 829.

but not strong enough of themselves to have any effect on blood pressure, are seen together with the Traube-Hering waves. The first of these reasons seems hardly applicable at least to the waves described by Traube as recurring at intervals of thirty seconds (Hering himself says the waves recur at the rate of 5 to 15 per minute). Concerning the second argument, slight spasmodic movements may often be observed in the limbs and trunk muscles of imperfectly curarized animals, and Knoll[1] has seen them recur at regular intervals, but they are far too slight to influence the blood pressure.

Mayer supports Hering's theory of irradiation from the respiratory centre on the grounds that any manipulation, as section of the vagi, which lowers the respiratory rate makes the waves more prominent; that stimulation of the central end of a cut vagus alters their type; and that they do not occur after paralysis of the respiratory centre through shutting off the blood supply to the brain. But these reasons are open to objection. Unless the lowering of the rate of breathing is accompanied by a corresponding increase in the depth of each respiratory movement — and the maintenance of the normal respiratory exchange after section of the vagi is doubtful — the accumulation of carbon dioxide should favor the occurrence of these waves; moreover, the vagi are concerned with the innervation of so many functions that to draw any positive conclusions regarding such an obscure subject from the effects of their section is hardly wise. The support drawn from the discovery that stimulation of the central end of the vagus affects the form of the wave, loses much of its weight in view of the fact that Knoll has since found that stimulation of any sensory nerve has the same effect. As to the third reason for his belief, Mayer seems to have forgotten that in occluding the blood supply of the medulla he paralyzed the vasomotor as well as the respiratory centre.

A better method of determining whether the respiratory centre is to be considered as a casual factor in the production of Traube-Hering waves would seem to be the use of some agent which paralyzes the respiratory and not the vasomotor centre. In some experiments with veratrum viride[2] I find that this substance offers us a means of thus separating the two functions. By proper doses (about 0.04 c.c. of the fluid extract per kilo, for dogs) the respiration

[1] Knoll: *Ibid.*, 1886, xcii, p. 447.

[2] Veratrum album has the same effect.

can be absolutely paralyzed while the rise of pressure brought about by the asphyxia demonstrates that the vasomotor mechanism is still functional. Under these circumstances I have seen the most marked rhythmical variations in the blood pressure, both when it was high (150 mm. Hg) and when it was low, either with artificial respiration or when the animal was asphyxiated. The *occurrence of Traube waves when the respiratory centre is paralyzed shows that the impulses which occasion their discharge do not arise in that centre.*

STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. IV.—LAWS OF CHEMOTAXIS IN PARAMECIUM.[1]

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1. Introduction.

IN the second paper of this series of Studies,[2] I have given an account of the mechanism of the reactions of Paramecium that requires a revision (for this animal) of the usual conceptions conveyed by the term chemotaxis. It was there shown that Paramecium reacts in essentially the same manner to all effective stimuli, and that the direction of motion in the reaction has no relation to the position of the source of stimulus, so that the animals are not directly attracted or repelled. When the animals are stimulated in any way, they swim backward, turn toward the aboral side, then swim forward over the new path so determined, without regard to the position of the stimulus. The terms positive and negative chemotaxis thus lose their content almost entirely, at least in the construction which is often put upon them. Nevertheless it will be convenient to employ these terms to express the fact that the Paramecia do, in the manner detailed in the paper above referred to, form collections in certain regions (positive taxis), and leave other regions empty (negative taxis). These phenomena retain their biological significance in full, whatever the means by which they are brought about. In view of the mechanism of the reactions, the following

[1] Scientific Results of a Biological Survey of the Great Lakes, directed by Jacob Reighard (Ann Arbor, Mich.), under the auspices of the U. S. Commission of Fish and Fisheries, No. 2. Published by permission of the Hon. George M. Bowers, Fish Commissioner.

[2] The following papers have appeared in the series of Studies of which this is the fourth: I. *Reactions to Chemical, Osmotic, and Mechanical Stimuli in the Ciliate Infusoria*, Journal of physiology, 1897, xxi, pp. 258–321. II. *The Mechanism of the Motor Reactions of Paramecium*, This journal, May, 1899, ii, pp. 311–341. III. *Reactions to Localized Stimuli in Spirostomum and Stentor*, American naturalist, May, 1899, xxxiii, p. 372. These papers will be referred to in the following as I, II, and III, respectively.

definitions may be given. Certain substances in solution set up in a Paramecium random movements, the direction of movement being changed at the time the stimulus begins to act and frequently so long as it continues to act. As a result of these undirected motions the Paramecium is in time, by the laws of chance, carried out of the region of the substance causing them, and prevented from re-entering. Hence toward these substances it may be said to be negatively chemotactic, and the substances may be called repellent. Other substances cause no motor reaction whatever, so that the Paramecia tend to come to rest within them, and these substances are further of such a nature that they throw the Paramecia into a peculiar physiological condition, such that coming in contact with a fluid not containing these substances causes the motor reaction above characterized, so that the animals are returned into the region of the substance causing no motor reaction. Hence if this region is small a dense assemblage of Paramecia may be formed within it. Substances of this character may therefore be said to be attractive, and the Paramecia are positively chemotactic toward them. The terms repellent and attractive, negative and positive chemotaxis, as used in this paper, are then to be understood to have significations in accordance with the above definitions.

2. Relation of Chemotaxis to Benefit or Injury.

The present paper deals with the nature of the substances thus causing or failing to cause the motor reactions, and with the laws of their action. The first question which will be taken up is in regard to the relation of chemotaxis to the benefit or injury to the organism, caused by the given substance. As shown in II, the effect of the motor reaction is on the whole to carry the organism out of the sphere of action of the substance causing the reaction and to prevent its returning. Is the production of this reaction due to the fact that the substance causing it is injurious to the Paramecia? This is a question of some interest in view of a common theoretical explanation of the origin of chemotactic movements. It is said that through natural selection such individuals as are repelled by injurious substances would survive and perpetuate their kind, while those not repelled by these substances would be destroyed; the converse being true as regards beneficial substances. We might then expect to find that the individuals which now survive have become accurately adjusted in these matters, being repelled by any substance

which injures them. Any injury might be conceived to result in the "organic analogue of pain," causing thus motor reactions which take the animal away from the source of injury.

We know already from the first of these Studies that the substances which cause the motor reaction most markedly, and hence give most clearly the phenomena of negative chemotaxis, are chiefly alkalies, while substances in which the Paramecia collect, giving the motor reaction only when they attempt to pass *out* of them, are substances having a weak acid reaction. Thus the distinction between repellent and attractive substances is, to a certain extent at least, based upon purely chemical differences. But it was shown in the same paper that many neutral salts likewise cause the reaction, so that the acid or alkaline character of the solution is not the only determining factor; further, that when acid solutions reach a certain strength, they too cause the same motor reaction as do other substances; it is only weakly acid solutions that are attractive, while stronger acid solutions are repellent. It is a well-known fact that organisms are often thus negatively chemotactic toward substances to which in a weaker solution they are positively chemotactic.

Now, what is the basis of this change of reaction with a change in the strength of solution? The solution of course becomes more injurious as it becomes stronger, and the answer to this question which lies nearest is that the negative chemotaxis of the organisms is due to the injuriousness of the solution. May we say that as soon as an acid solution becomes equally injurious with the repellent alkaline solution, it too becomes repellent? And can we generalize the answer and say that the animals are throughout negatively chemotactic to certain substances in virtue of the fact that these substances are injurious to them?

If the injurious quality of the substance is the determining factor in causing the reactions which result in negative chemotaxis, then evidently two substances which are equally injurious must have equal powers of repelling Paramecia, and if one substance is injurious in a weaker solution than a second, then also must the first substance be repellent in a weaker solution than the second. In other words, the repelling powers of the two substances must be proportional to their injurious effects.

This gives a method of testing the question. We may determine the weakest solutions of two or more substances that repel the Paramecia, and note the ratio of their strength; then determine the

weakest solutions of the same substances that will kill the Paramecia in a given time, noting their ratios as before. If the two ratios are approximately the same — that is, if the repellent powers are proportional to the injurious effects — then the evidence is in favor of the proposition that the production of the motor reaction resulting in repulsion depends upon the injurious effect of the substances; if the ratios are not the same, then the relation between repellent power and injuriousness is not one of direct dependence.

I have compared in this way a considerable number of substances. The repellent powers of different chemicals were tested in the manner described in I and II for studying chemotaxis, — by introducing with a pipette drawn to a capillary point a drop of the substance beneath the cover-glass of a slide of Paramecia. The substances to be tested were dissolved in proper proportions in distilled water. In distilled water alone the Paramecia form collections (as shown in I), so that the repellent powers of the solutions are due wholly to the dissolved substance. For finding approximately the weakest solution of a substance that causes repulsion, it is convenient to proceed as follows: A series of covered watch-glasses are filled with solutions of the given substance, each successive watch-glass with a solution one half or one fourth as strong as that in the preceding one. Thus we shall have perhaps the following solutions: $\frac{1}{5}$, $\frac{1}{20}$, $\frac{1}{80}$, $\frac{1}{320}$, and $\frac{1}{1280}$ per cent, etc. The Paramecia are placed on a slide beneath a long cover-glass supported at the ends by bits of glass tubing. Now with the capillary pipette drops of solutions of different strengths are introduced beneath the four corners of the cover-glass, — under one corner a drop of $\frac{1}{5}$, under the next $\frac{1}{20}$, then $\frac{1}{80}$, and $\frac{1}{320}$ per cent. We shall probably find that some of the drops remain empty, while others are quickly filled with Paramecia. Suppose that the $\frac{1}{5}$, $\frac{1}{20}$, and $\frac{1}{80}$ per cent remain empty, while the $\frac{1}{320}$ per cent is quickly filled. It is thereupon evident that the weakest solution which repels the Paramecia lies somewhere between $\frac{1}{80}$ and $\frac{1}{320}$ per cent. By making proper intermediate grades between these two, approximately the weakest solution that repels the Paramecia can usually be determined very expeditiously. All the precautions insisted upon in I as necessary for carrying on such experiments were of course rigorously observed.

I will present in detail the results in two typical cases, then summarize my results on the sixty-five substances tested, in the form of a table.

For a first case we may take two chemically related substances, toward both of which the Paramecia show positive chemotaxis when the solutions are very weak. Chromic acid and potassium bichromate fulfil these conditions. These substances both have an acid reaction, due to the same component in each case, and the Paramecia gather in weak solutions of both of them. In stronger solutions both cause the motor reaction which results in repulsion. Is this due in each case to the injurious effect of the stronger solutions?

For potassium bichromate the weakest solution that repels the animals is about $\frac{1}{20}$ per cent. For chromic acid the weakest solution which sets the motor reaction in operation is about $1\frac{1}{50}$ per cent. The ratio of repellent powers is therefore 7.5 to 1 in favor of the chromic acid.

The injurious effects of the solutions are now tested as follows: Watch-glasses with solutions of different strength are arranged in series, as above described, and into each a drop of water swarming with Paramecia is introduced and quickly mixed. The solutions are made in such a way that after the introduction of the water containing Paramecia they are of the recorded strength. The weakest solution in which all the Paramecia are killed in one minute is thus determined for the two substances.

For the potassium bichromate it is found in this way that the weakest solution which kills the Paramecia in one minute is a one per cent solution, — that is, a solution twenty times as strong as the weakest solution that repels them. In the chromic acid they die somewhat more quickly in a $1\frac{1}{50}$ per cent solution, — a solution of the *same* strength as the weakest which causes repulsion. The ratio of the injurious effects of the two substances is therefore as 150 to 1 in favor of the chromic acid.

Comparing the two ratios, we find that they are strikingly unequal. While chromic acid has but $7\frac{1}{2}$ times the repellent power of potassium bichromate, it is 150 times as injurious. Potassium bichromate repels in a strength $\frac{1}{20}$ of that which is severely injurious; the chromic acid does not repel until it has reached a strength which is already destructive. In the latter case the repellent power is evidently due directly to the injurious effects; repellent power and injurious effects appear at the same point as we pass from weaker to stronger solutions. If the conduct of the Paramecia is observed under the microscope as they swim backward after coming in contact with a drop of $1\frac{1}{50}$ per cent chromic acid, it is at once evident that they are

decidedly injured, and frequently they die after swimming backward a little distance. The motor reaction is thus not set in operation by the chromic acid until the Paramecia are already injured and it is too late to save them. When such a drop is introduced beneath the cover-glass, the Paramecia are therefore killed by scores and the drop is soon surrounded by a ring of the dead animals. If a drop of a solution somewhat weaker than $\frac{1}{150}$ per cent is introduced, the Paramecia enter it directly, forming a dense gathering, and after a time it may be observed that many individuals of the group are dead or severely injured. In the case of the potassium bichromate, on the other hand, the Paramecia are never injured whatever the strength of solution used; they gather in a ring about a drop of strong solution, but do not enter it deeply enough to be injured.

For these two substances then the test results negatively; potassium bichromate has twenty times the repellent power it should have if repellent power depends alone on the injuriousness of the solutions.

We may next test two substances which do not attract the Paramecia in any strength, and which are not injurious in virtue of their chemical properties at all, but only as a result of their osmotic pressure. As such substances we may select sodium chloride and cane sugar. Repeated comparative tests with isotonic solutions of these two substances indicate that neither is immediately injurious to the Paramecia except through osmotic pressure; strong isotonic solutions of the two substances kill the Paramecia in approximately the same period of time. Thus if Paramecia are introduced at the same time into 1 per cent sodium chloride solution and 9.64 per cent cane sugar (which is isotonic with 1 per cent solution of sodium chloride), in both cases nearly all the individuals are found to be dead twenty minutes after they were introduced, and in neither solution does the proportion of dead markedly preponderate. This result for Paramecium differs from that gained by True[1] for Spirogyra; in the case of Spirogyra sodium chloride was found to have a distinct toxic effect in addition to its osmotic properties.

The weakest solution of sodium chloride which repels the Paramecia is from $\frac{1}{10}$ to $\frac{1}{20}$ per cent. The weakest solution of cane sugar which sets the motor reaction clearly in operation is 10 per cent. Taking the repellent solution of sodium chloride as $\frac{1}{10}$ per cent, the

[1] True: Botanical gazette, 1898, xxvi, p. 407.

repellent power of sodium chloride is to that of cane sugar as 10 to $\frac{1}{10}$, or as 100 to 1. On the other hand, as stated above, 1 per cent sodium chloride has the same injurious effect as 10 per cent cane sugar, so that the injurious effects of sodium chloride are to those of cane sugar as 10 to 1. The repellent power of the cane sugar, like that of the chromic acid, is plainly due to its injurious properties. The motor reaction is not set in operation until plasmolysis has begun, as shown by the evident shrinkage of the bodies of the animals, and it is usually too late at this time to save them from destruction. If a drop of strong sugar solution is introduced into a slide of Paramecia, it soon becomes filled with the dead Protozoa. On the other hand a drop of sodium chloride is not destructive whatever its streagth, since the motor reaction is set in operation long before the Paramecia have entered deeply enough to be injured. Sodium chloride has, as shown by the ratios given above, ten times the repellent power that it should have if repellent power depends entirely upon injurious effects.

A large number of compounds, chiefly inorganic, were tested as to the relation between repellent power and injurious effects, and it was found that the cases given above are typical for all. We can divide chemical substances as regards this matter into two classes, as shown in the following table. In the first column may be placed together substances in which, as in potassium bichromate and sodium chloride, the repellent power is great in proportion to the injurious effects. These substances all set the motor reaction in operation long before any injury has taken place, so that the Paramecia are not harmed in the least when a drop of one of them is introduced into a slide preparation of the infusoria. In the other column may be placed substances in which, as in chromic acid and cane sugar, the repellent power is slight in relation to the injurious effects; the motor reaction in all these substances is set in operation only when the Paramecia are already injured and it is too late to save them. A drop of any one of these substances introduced beneath the cover-glass of a slide preparation of Paramecia proves exceedingly destructive.

We may for convenience classify the substances in the table also into those which are attractive to the animals in weak solutions (A), and those which are repellent in all effective solutions (B).

TABLE I.

1. Repellent power strong in proportion to injurious effects: chemotaxis protective.	2. Repellent power very weak in proportion to injurious effects: chemotaxis not protective.
(A. *Attractive Substances.*)	
Sodium fluoride, Potassium bichromate, Ammonium bichromate, Potassium ferricyanide.	HF, HCl, HBr, HI, H_2SO_4, HNO_3, Acetic Acid, Tannic Acid, Picric Acid, Chromic Acid, Potassium fluoride, Potassium permanganate, Ammonia alum, Ammonio-ferric alum, Chrome alum, Potash alum, $CuSO_4$, $CuCl_2$, $Cu(C_2H_3O_2)_2$, $ZnCl_2$, $HgCl_2$, $AlCl_3$.
(KBr), (KCl), (RuCl), (CsCl).[1]	
(B. *Repellent Substances.*)	
LiCl, NaCl, (KCl), (RuCl), (CsCl),[1] LiBr, NaBr, (KBr), RuBr, LiI, NaI, KI, RuI, Li_2CO_3, Na_2CO_3, K_2CO_3, $LiNO_3$, $NaNO_3$, KNO_3, NaOH, KOH; $KBrO_3$, NH_4F, NH_4Cl, NH_4Br, NH_4I, $CaCl_2$, $SrCl_2$, $BaCl_2$, $Ca(NO_3)_2$, $Sr(NO_3)_2$, $Ba(NO_3)_2$.	Cane sugar, Lactose, Maltose, Dextrose, Mannite, Glycerin, Urea.

From this table and the foregoing discussion it is evident that the question proposed is to be answered in the negative. The injuriousness of a substance is not the determining factor in its repellent power, since the repellent power of different substances is not at all proportional to the injuriousness of their solutions. In the substances of the second column, such power as they have to set in operation the motor reaction of Paramecium is indeed evidently due directly to the injuries caused, though this power is nothing like so precise and strong as in the substances of column 1, not being sufficient even to prevent the Paramecia from entering and being destroyed.

The fact that the injuriousness of a solution is not the chief determining factor in its repellent power seems to indicate that the common theory, according to which the reactions of organisms have been fixed by natural selection through the destruction of the individuals not repelled by injurious substances, does not contain the whole truth.

[1] In the case of the substances enclosed in parentheses, the reactions of Paramecia from different regions varied, as described in a subsequent part of this paper.

Probably many of the substances experimented with have never been met by the Paramecia in the course of their evolution, and their reaction toward these must be determined by other laws. Possibly their reactions are adjusted by natural selection to some small number of substances, while to other substances they react by analogy as it were, — by the similarity of their effects to those of the substances to which the reaction has been adjusted by natural selection. Thus it may be that repulsion for some alkali plays an important part in the biology of the animals, and that the reaction toward other substances of this sort is due to their similarity to the primary one. We know that to the tendency to collect in weak solutions of carbon dioxide is due the apparently gregarious habit of the Paramecia, and this probably serves a useful purpose somewhere in the life of the animals. It may be that the tendency to gather in other acid solutions, even such destructive ones as sulphuric acid and copper sulphate, is a sort of reflection of this tendency to collect in carbonic acid, and that natural selection has had no opportunity to correct this tendency directed toward injurious substances. That there is much opportunity for natural selection to act is clear when one tests any chemical substance by bringing a drop of it among a large number of Paramecia. There is always a number of individuals who do not give the typical reaction, some being much more and some much less sensitive than the majority.

Another consideration of importance is the following: It may not be at all the *direct* effects of a substance on the Paramecia, through which natural selection has adjusted the reaction toward that substance. Thus it may be that in solutions of a certain nature, harmless in themselves, the Bacteria which form the food of the Paramecia are never found; natural selection would then act by starving such Paramecia as were not repelled by these solutions.

3. Relation of Chemotaxis to Chemical Composition.

If the injuriousness of substances is not the determining factor in their repellent power, what *is* the determining factor? An examination of the table above given brings out the following facts. All the substances in which the repellent power is relatively great contain a large proportion of some metal of either the alkali group (Li, Na, K, Ru, Cs, or NH_4) or of the earth alkali group (Ca, Sr, Ba). This is true of all, whether having an acid, a neutral, or an alkaline reaction. On the other hand most of the substances in the second column con-

tain none of these elements. It is true that certain of these compounds, as potassium fluoride, potassium permanganate, and the alums do contain members of the alkali group. But in potassium fluoride the qualities of the compound are due almost entirely to the fluorine component, as is shown by the fact that this salt, like hydrofluoric acid itself, attacks glass energetically. In potassium permanganate and the alums the proportion of alkali metal is small, as is shown by their formulas. Thus the formula for potash alum is $KAl(SO_4)_2 + 12\ H_2O$, so that in the molecule composed of forty-eight atoms but one of these is K; the properties of these compounds are very little due to the alkali component. The proportion of alkali metal is very much larger in the substances of the first column. This dependence upon the presence and proportion of an alkali metal is in practice so striking that in working with salts whose action on the Paramecia is not yet known, it is usually possible to predict with certainty from the chemical formula into which column they will, upon experiment, be found to fall.

The results on these sixty-five compounds appear then to justify the following conclusion: Compounds which contain a large proportion of one of the alkali or earth alkali metals set in operation the motor reaction of Paramecium even when exceedingly weak; their repellent power is therefore great in proportion to their injurious effects, and the infusoria are not endangered by the presence on the slide where they are swimming of a drop of a solution of these compounds. Substances which do not contain a large proportion of one of these metals as a rule set in operation the motor reaction of Paramecium only when the animals are already injured; their repellent powers are very weak in proportion to their injurious effects, and a drop of one of them introduced into a preparation of Paramecia is very destructive to the latter.

The fact, however, that the injurious properties of a substance on reaching a certain intensity of action do produce the motor reaction is worthy of special notice; in all the substances in the second column the reaction is manifestly due directly to the injuries inflicted, without regard to the nature of the substance. Paramecium is not sufficiently sensitive to injuries to make the reaction thus produced of any protective value, at least under experimental conditions. But it may easily be conceived that this sensitiveness might be so increased that the reaction would be of the greatest importance for preserving the animals; it is probable that in many organisms this condition is real-

ized. We seem to have in Paramecium the very lowest step in the production of reactions due to the "organic analogue of pain" (to use a phrase employed by J. Mark Baldwin), — a step so low that the power of thus reacting seems to have no value for the life of the organisms.

The chief determining factor in the repellent power of solutions is then, for Paramecium, of a chemical nature, with little relation to the comparative injuriousness of the solutions.

The conclusion above drawn as to the repellent power of the alkali metals is reinforced and extended by certain results gained in a study of the compounds of these metals with the halogens, — compounds of lithium, sodium, potassium, rubidium, and cæsium on the one hand, with fluorine, chlorine, bromine, and iodine on the other. These form a definitely circumscribed group of compounds, the characteristics of which, as is well known, vary in many respects with the molecular weights of the elements composing them. A qualitative and quantitative study of the reactions of the Paramecia toward these compounds was begun at Put-in-Bay Island, Lake Erie, during the summer of 1898, in the hope that such a study would throw light on the essential nature of chemotaxis. The work was not finished at Put-in-Bay, and on taking it up again at Hanover, N. H., it was found that the Paramecia here gave in part different reactions from those studied at Put-in-Bay. The differences are of such a nature as to be not unintelligible, yet it will be necessary to consider separately the results obtained at the two places.

The elements belonging to the group of halogens may be arranged in a series, beginning with that having the least atomic weight and proceeding to that which has the greatest atomic weight, as follows: fluorine (at. wt. 19), chlorine (at. wt. 35), bromine (at. wt. 80) iodine (at. wt. 127). As is well known, this is also the order of the chemical activity of these elements, the lightest being most active. In the same way the alkali metals may be arranged in a series beginning with the lightest, as follows: lithium (at. wt. 7), sodium (at. wt. 23), potassium (at. wt. 39), rubidium (at. wt. 85), and cæsium (at. wt. 133). This series also gives in a general way the order of chemical activity of the elements, the lighter ones being more active.

The compounds of these two sets of elements may be arranged in a table in such a way as to give the halogen series from above downward, the alkali series from left to right. In the ensuing table,

arranged in this manner, are placed only the compounds which I was able to procure and use for studying the reactions of the Paramecia at Put-in-Bay. For convenience the hydroxides of the metals and the acids of the halogens are brought into the table, — the former constituting the lower row, the latter the right-hand column.

TABLE II.

	Li(7)	Na(23)	K(39)	Ru(85)	Cs(132)	
F(19)	....	NaF	KF	....	....	HF
Cl(35) . . .	LiCl	NaCl	KCl	RuCl	CsCl	HCl
Br(80) . . .	LiBr	NaBr	KBr	....	....	HBr
I(127) . . .	LiI	LiI	KI	....	....	HI
		NaOH	KOH			

In this table, as will be observed, the compounds of the lighter (and more active) metals are placed to the left; the compounds of the lighter (and more active) halogens in the upper row. In the right-hand upper part of the table are given therefore compounds of light, active halogens with heavy, inactive metals; in these the influence of the halogens might be expected to predominate. In the left-hand lower corner are given compounds of light, active metals with heavy, inactive halogens; in these the metal component might be expected to predominate.

Now some of these substances repel the Paramecia in any effective concentration; that is, they produce the motor reaction which results in carrying the Paramecia outside of their influence; these we may call the repellent substances. Others when weak do not cause the motor reaction when the Paramecia enter them, but when the animals attempt to swim *out* of the drop into the surrounding water the motor reaction occurs, and the Paramecia return into the drop. They thus form collections either within such drops or surrounding them in a close ring, — in the manner described in I, p. 269. These we may call attractive substances.

All the attractive compounds in the above table are underscored; those not underscored are repellent. It will be seen, first, that all the

acids or hydrogen compounds of the halogens are attractive, the hydroxides, or hydroxyl compounds of the metals repellent. Further, that all the compounds of the lightest, most active halogen (fluorine) are attractive. Of the next lightest, chlorine, three salts are attractive and two repellent. Of the heaviest, most inactive halogen, iodine, all the salts studied are repellent. Of the lightest, most active metal, lithium, all the salts tested are repellent, and the number of repellent salts decreases as we pass toward the heavier metals. Thus the compounds of light, active metals with heavy, inactive halogens are repellent; those of light, active halogens with heavy, inactive metals are attractive. A diagonal line can therefore be drawn, as shown in the table, from the upper left hand to the lower right hand, separating all the attractive substances on the right from all the purely repellent substances on the left.

It thus appears that the Paramecia react to these compounds with relation to the elements of which they are composed. The metal components tend to repel the Paramecia; the halogen components to attract them. The acids of the halogens, in which the halogen may be presumed to give character to the compound almost alone, do not repel except in such solutions as to be immediately destructive, as previously discussed. The hydroxides, or bases, in which the metals give the character to the compound, are repellent in any solution strong enough to produce any effect whatsoever. Those compounds which are partly metal and partly halogen are attractive or repellent in accordance with whichever one is the predominant element.

The same conclusion is reached by a study of the exact method of behavior of the Paramecia toward the substances above classed as attractive. Toward these compounds the infusoria are as a rule *both attracted and repelled*. As just stated the acids are purely attractive, the bases purely repellent. Toward potassium fluoride, in which the halogen component is so active as to attack glass even in this salt, the animals react as toward a pure acid. Toward sodium fluoride, in which the fluorine is so bound by the sodium that the two produce a comparatively inactive salt, the Paramecia are both strongly attracted and strongly repelled. When a drop of one per cent sodium fluoride solution, for example, is introduced into a slide of Paramecia, it will soon be found that the animals have gathered in a dense ring about the drop, leaving its centre entirely free. In this ring they swim back and forth, giving the motor reaction whenever they come

to the inner side of the ring, where the stronger sodium fluoride is, or when they come to the outer side of the ring, where pure water is. Sodium fluoride thus gives a striking example of apparently positive and negative chemotaxis to the same substance. Of compounds outside the group at present under consideration, potassium and ammonium bichromate and potassium ferricyanide show precisely the same phenomena.

The other salts classified in the table as attractive show the same phenomena as sodium fluoride, except that the attraction is less strong and the repulsion stronger. The Paramecia gather in a narrow ring about them, and this ring contains at a given time comparatively few individuals, though enough to make the ring evident as a rather sharply defined white line around the drop. If the individuals constituting this ring are closely observed, it becomes evident that the motor reaction is set strongly in operation when they come to the inner boundary of the ring, where the stronger solution of the salt is found, and much less strongly when they come to the outer boundary, against the ordinary water. Thus many individuals remain in the ring but a short time, finally crossing the outer boundary and escaping, so that the ring does not increase continually in numbers and size, as it does in the case of sodium fluoride; it consists of changing individuals, each one being detained usually but a short time. In some of the salts the ring thus formed is almost evanescent: for a short time it will be distinct, then almost disappear, then reappear again. Finally, about the salts which are not underscored in the table no ring of Paramecia is ever formed.

This formation of a ring of greater or less extent about solutions of various salts is a very curious phenomenon. The Paramecia are evidently thrown into such a condition by a very weak solution of the substance that the contact with the surrounding water now acts as a shock or stimulus to set the motor reaction in operation, — while this motor reaction is also induced by a stronger solution of the same salt, such as is found nearer the centre of the drop. In view of the facts adduced, it seems apparent that the shock on reaching again the outer water is due to the physiological effect of the halogen component, as this shock alone is produced in compounds in which, as in acids and potassium fluoride, the halogen is the element that chiefly gives character to the solution. On the other hand, the stimulus due to entering the salt itself is a result of the metal component, since this stimulus alone is produced in those compounds in which, as in

hydroxides (and carbonates) the characteristics of the compound are due chiefly to the metal.

The above results were obtained at Put-in-Bay in a series of about 1000 tests of the substances in the table. The compounds used were tested many times, dissolved both in distilled water and in the same water in which the Paramecia were found, with every possible precaution to secure accuracy, and with uniform results as given above. But on continuing the series of experiments at Hanover, N. H., with the intention of filling the gaps in the table as above given, different results were obtained with the Paramecia of this region for certain of the salts. Toward KCl, KBr, RuCl, and CsCl the Paramecia of Hanover show only repulsion, without any sign of the formation of a ring about them, which was so evident at Put-in-Bay. Toward the NaF and KF on the other hand they react exactly as did those at Put-in-Bay, forming a ring about the NaF and reacting toward the KF as toward an acid. Toward all other members of the above table the Hanover Paramecia likewise react exactly as did those at Put-in-Bay. This variation is evidently not of a character to modify in any way the conclusions to be drawn. The Paramecia at Hanover simply fail to show a very faint reaction given by those from Lake Erie in the presence of certain substances; they seem therefore merely slightly less sensitive; in other respects the reactions are similar in all the Paramecia studied. The Paramecia at Put-in-Bay were cultivated in lake water containing decaying water plants, chiefly Nuphar, Naias, and Ceratophyllum. At Hanover the Paramecia came from cultures of hay, in water from small ponds of the neighborhood. Possibly the difference in the culture medium has something to do with the difference of reaction in the Paramecia of the two regions.

The reactions toward certain other substances may be stated briefly. Toward the carbonates of lithium, sodium, and potassium the reaction is the same as toward the hydroxides of these metals. Also toward the nitrates of these metals and toward the chlorides and nitrates of calcium, strontium, and barium the reaction is of the nature of repulsion alone, as toward the iodides, sodium chloride, etc., given in the table. Toward salts of the heavy metals, as cupric chloride, cupric sulphate, mercuric chloride, zinc chloride, aluminium chloride, the Paramecia are attracted, as toward acids.

From the facts given in the above table and discussion the following conclusions may be deduced: —

1. The base-forming elements of the alkali and earth alkali groups when present in a compound tend to produce the motor reaction in Paramecia whenever the animals come in contact with these compounds, even in very dilute solutions. This may be put in terms of the modern theory as follows: the kations of the alkali and earth alkali groups have a strong tendency to induce the motor reaction in Paramecia. As a consequence these infusoria are kept from entering such solutions even when the solutions are very weak.

2. The ions of the acid-forming elements, such as the halogens, and in fact the anions of *all* acids, do not themselves tend to cause the motor reaction in Paramecia, except when present in such strength as to be immediately destructive. On the contrary, they tend to throw the Paramecia into such a physiological condition that contact with any solution containing no anions produces the motor reaction. As a consequence, Paramecia tend to congregate in regions where it is mainly the anions that give character to the solution.

The fact that the same result as for acids is obtained for salts in which the anion is much the most powerful component, as in potassium fluoride, shows of course that the results with acids are not due to the hydrogen ion of the acids.

3. Compounds that contain both kations of the alkali elements, and anions, may give a combination of these results, — a tendency to cause the motor reaction (with its reversal of cilia) both when the animals enter the solution (due to contact with the kations), and when they leave the solution (effect of the anions). In this case the Paramecia collect in a ring about a drop of the compound.

4. Salts that contain the relatively inactive kations of one of the heavy metals, as aluminium, copper, zinc, mercury, produce an effect due only to the anion, hence their effect is like that of an acid.

These conclusions in regard to the different effects of the anions and kations on the motor reactions of Paramecium certainly seem to invite a discussion of the relation of the activities of the ions during the passage of an electric current through a solution to the movements of the Paramecia under the same conditions. As is well known, when a continued current is passed through the water containing Paramecia, they all swim with one accord to the kathode, — thus in the same direction as that in which the metallic ions are moving. It is difficult to resist the impression that there must be some such relation between the movements of these ions and the movements of

the organisms as has been suggested by Loeb and Budgett.[1] Yet a discussion of this matter will hardly lead to valuable results until it can be accompanied by a careful re-examination of the phenomena of electrotaxis, with especial reference to the relation of the electrotactic movements to the general motor reaction of Paramecium described in the second of these Studies. We possess already a detailed account of the movements of the cilia in electrotaxis, due to Ludloff,[2] but these observations, as well as my own observations on electrotaxis, published in I, were made before the general plan of the motor reactions of Paramecium was known, so that a re-examination might lead to the discovery of facts of fundamental significance.

4. Relative Repellent Power of Related Substances.

In the first part of this paper I have shown that with relation to their action upon Paramecium, chemical substances are divisible into two classes, in one of which the repellent power is very great in proportion to the injurious effects, while in the other class such slight repellent power as exists is due directly to injuries caused. In this latter class a quantitative study of the relative repellent powers of the different members shows of course that the power of setting in operation the motor reaction of Paramecium is in a general way proportional to the injuriousness of the solutions. But to what factor are the relative repellent powers among themselves in the members of the *first* group due? I have already shown that the relative repellent power of members of the first group, as compared with that of members of the second group, is due to the presence in preponderating amounts of some metal of the alkali or earth alkali series. But this leaves untouched the question of the relative repellent powers of members of the first group among themselves. For example, will sodium chloride be more or less repellent than potassium chloride, and why?

On the question of the relative repellent powers of different members of this group much labor was spent, with rather unsatisfactory results. This is due to the fact that the different compounds have specifically different ways of affecting the Paramecia, some modifying the general motor reaction in one way, some in another, — although never changing the essential character of the reaction. This is a matter worthy of special study. Chemotaxis is a complex pheno-

[1] Loeb and Budgett: Archiv f. d. ges. Physiol., 1897, lxv, p. 518.

[2] Ludloff: *Ibid.*, 1895, lix, p. 525.

menon, the motion to or from a substance being the indirect resultant of the specific physiological effects of this substance upon the different phases of the motor reaction. These specific physiological effects are therefore the primary phenomena. This fact was not recognized at the time my work was done, so that full records were not made on these points. On a few substances observations were made, which I will give here as examples. As shown in II, the typical motor reaction is as follows: (1) the cilia are reversed, causing the Paramecia to swim backward; (2) the aboral cilia of the anterior half of the body strike transversely toward the oral side, turning the animal toward the aboral side; (3) the cilia all strike backward as at first, carrying the animal forward over a path which lies at an angle to the original path. This reaction is given in characteristic form when the animal comes in contact with a weak alkali; all the parts are fairly though not excessively pronounced. On coming in contact with a strong acid solution, the Paramecia dart furiously backward a long distance. Then they turn and dart furiously forward, so that they often thus rush directly into the drop of acid, and perish. If their course after turning, however, tends to carry them away from the acid, when they strike the water into which no acid has diffused they react again by reversing and turning, — showing that the acid has put them into a peculiar physiological condition. Some acid substances cause the reaction with certain of its typical features nearly or quite suppressed. Thus when the animals swimming forward come in contact with diffusing mercuric chloride, they dart straight backward, the anterior end being curved a little to one side; at the same time the body begins to swell. Then they start straight forward again, the turning usually being nearly or quite omitted. Paramecia which come in contact with a drop of $\frac{2}{3}$ per cent calcium chloride solution while swimming forward, pass in without hesitation, but are excited and the forward course is hurried, so that they soon pass out. They thus never remain quiet in the calcium chloride, and as a result it remains nearly empty, the Paramecia gradually settling down elsewhere. Yet one cannot say that the calcium chloride repels the animals; it merely excites them, without causing even a change in the direction of motion. Paramecia introduced directly into 2 per cent barium nitrate solution are paralyzed instantly. But in three or four minutes they revive, and swim slowly about. In 2 per cent calcium nitrate solution, on the other hand, they are at once violently excited, swimming forward and backward swiftly. In solution of

strontium nitrate they are likewise excited, but much less so. In 1 per cent sodium bromide and sodium iodide they first dart backward, then turn, then swim forward, then backward again, — repeating the entire typical motor reaction many times. By 1 per cent potassium iodide each part of the motor reaction is almost indefinitely prolonged; they swim backward perhaps ten minutes at a time, then they turn toward the aboral side, and *keep on turning*, whirling thus on the short axis for twenty minutes or more. In 10 per cent solution of cane sugar they at first give no reaction; gradually as plasmolysis occurs they begin to swim backward, then turn, then swim forward, repeating this, as in sodium bromide solution. It will readily be seen in view of these peculiarities in reaction, that it is not always possible to classify the conduct of the Paramecia at once into the two categories of attraction and repulsion.

Moreover, toward many compounds there exist great individual differences among the Paramecia as to the reaction given; at times a few individuals will swim directly into a solution, while the rest leave it empty. There is thus no absolute criterion for determining at what strength the repulsion of a solution really ceases. Moreover, the sensitiveness of the Paramecia to solutions of different strength of a given substance seems to vary according to something like Weber's law, — so that the least observable differences in reaction are between that due to a solution of given strength, and that due to a solution of twice this strength. It is often difficult to determine, for example, whether the repellent power of a substance ceases at $\frac{1}{4}$% or $\frac{1}{8}$% per cent; but upon this decision may depend our conclusion as to which of two substances shows the greater repellent power.

The following results upon certain salts are, however, based upon a very large number of experiments, and so far as they go, may be given with confidence. The method of experiment was that already described, the two substances being compared always under exactly similar conditions in all respects, by introducing a drop of each beneath the cover-glass of the same slide of Paramecia. A special study was made in this way of the halogen salts of the alkali metals, — the chlorides, bromides, and iodides of lithium, sodium, potassium, rubidium, and ammonium, together with the chloride of cæsium. This study was made at Hanover, and therefore upon Paramecia that were repelled by all these salts, so that the repulsion was not confused with any accompanying attraction.

As explained in connection with certain similar results given in I,

it is not possible to give a figure which shall represent in a general way the weakest concentration of any one of these substances that causes repulsion in the Paramecia, on account of the very great variation in the reaction of Paramecia from different cultures and under different conditions. For example, on testing Paramecia from two different cultures at the same time, it was found that those from one culture were decidedly sensitive to lithium chloride in a $\frac{1}{4}$ per cent solution, leaving a drop of this strength empty for a long time, while those from the other culture were quite indifferent to the same chemical even in a strength sixteen times as great, swimming in and out of a drop of $\frac{2}{5}$ per cent solution of lithium chloride without reaction. But the *relative* sensitiveness of Paramecia to two or more different chemicals seems to remain fairly constant, so that the order of sensitiveness to different chemicals can be determined. I give therefore the order of sensitiveness to the different metal compounds of each halogen, — the test of sensitiveness being the relative strength of solution of the different substances that causes repulsion.

For the chlorides the order is as follows, beginning with the most repellent: LiCl, KCl, NaCl, RuCl, CsCl. That is, the repellent power decreases as the atomic weight of the metal component increases, except that the sodium compound is moved down one place, being less repellent than the compound of the heavier potassium. The weakest repellent solution varies from about $\frac{1}{4}$ per cent in the lithium chloride to about $\frac{1}{2}$ per cent in the chloride of caesium.

For the bromides the order is, beginning with the most repellent, LiBr, KBr, NaBr, RuBr; for the iodides LiI, KI, NaI, RuI. In these two sets of salts the order is thus the same as for the chlorides. In general, therefore, the repellent powers of the halogen salts of the alkali metals varies inversely with the atomic weight of the metal components, except that in each case the sodium compound is less repellent than the heavier potassium compound. The ammonium salts were found in each case to possess almost exactly the same repellent power as the corresponding salts of potassium.

Neglecting for the moment the inverted order in the potassium and sodium salts, two causes suggest themselves for the inverse proportion between the repellent power and atomic weights of the elements. The halogen component remaining the same throughout a given series, the molecular weights of the salts of course increase with the atomic weights of the metal components. Hence the less repellent compounds have the greater molecular weight, so that a

solution of given strength by weight of one of these salts contains fewer molecules than does the corresponding solution of one of the lighter salts. Supposing the molecules to have throughout the same repellent power, the solution of the lighter salt, containing more molecules, will of course be more repellent. A second cause may be the usual greater chemical activity of lighter elements. If both these causes are efficient factors, they of course reinforce each other in the series above given. The reason for the anomalous position of the sodium salts, as weaker than the potassium salts, does not appear.

On comparing the series of the different halogen salts of a given metal, as NaCl, NaBr, NaI, I have found it impossible, in spite of much labor directed upon this point, to determine satisfactorily the relative repellent powers. Apparently the three salts have almost exactly equal repellent powers, and many sets of experiments upon the corresponding series for each of the four metals, lithium, sodium, potassium, and rubidium, have not made it possible to differentiate them. In every case the repulsion was very nearly equal for all, and the slight differences observed in one set of experiments did not hold for the next. It is evident that the two factors given above as possibly reinforcing each other in causing the greater repellent power in the lighter salts of the metal series of a single halogen, would, in the halogen series of a single metal, work in opposite directions. The greater number of molecules in the lighter compounds (as of chlorine) would tend to produce greater repulsion. But the greater chemical activity of the lighter chlorine component would tend to neutralize the effect of the metal component, to which alone the repulsion is due. Possibly it is to this interference that the approximately equal repellent power of the chlorides, bromides, and iodides of a given alkali metal is due.

Much time was spent on a similar quantitative study of the nitrates of the alkali metals and of the earth alkali metals, but without clearly defined results. The different nitrates have specifically different effects on the Paramecia; the repellent power is the resultant of several factors, any one of which may vary independently of the others. Thus the phenomena differ in the different salts, and there is no certain criterion for comparing the repellent powers of two salts having different specific physiological effects.

The above somewhat fragmentary and partially negative results of quantitative studies are given, not only because the data obtained are of themselves of some value, but also because it is of importance to

show which lines of investigation promise to be fruitful and which do not. This method of study has thrown chiefly a negative light upon chemotaxis, — showing what it is *not*. The evidence that chemotaxis is not a direct simple phenomenon, due to simple forces of attraction and repulsion, is in itself of importance.

The foregoing study of laws of chemotaxis in Paramecium, while it is believed to contain some results that are new and significant, is not put forth as in any sense exhaustive. Certain laws came forth clearly, while certain lines of investigation proved comparatively unfruitful; these I have set forth for the positive value of the one and the negative value of the other. The work has been done chiefly on inorganic compounds; almost the whole field of organic compounds remains to be examined. Certain facts in the reactions toward inorganic substances remain at present isolated, not falling under any of the general laws above given; thus certain experiments seem to show that there is a strong repulsion exhibited toward iodine, bromine, and chlorine when uncombined, — though in the ionic condition these elements are purely attractive. The effect of metals other than the alkalies and alkaline earths, when uncombined with a strong acid, is of interest, and has not been determined. Finally, an examination of the specific physiological effects of chemical compounds on the different parts of the motor reaction, as well as on the substance and the general functions of the animals, would be of fundamental value. Such a study would not be directed purely upon one aspect, as upon repulsion and attraction, which are really the resultant of complex factors, but should have in view any change produced by the substances studied

Summary and Conclusions.

In the introduction to the first of the Studies of which this is the fourth, the program for work was given as the investigation of the life activities of a single unicellular organism as completely as possible, in the hope that when such a study should have been carried to some degree of completeness, it should furnish a key to the understanding of the activities of other organisms, and should throw light on the essential nature of chemotaxis and similar phenomena. The investigation of the activities of Paramecium thus begun is far from being complete, yet I believe it may be said that the results thus far gained have already justified the plan of work. The mechanism of attractions and repulsions has been made out, and the third paper of the series shows that this furnishes at once a key to the understanding of

the activities of certain other infusoria (Spirostomum and Stentor). Studies now in progress show that it throws equal light upon the activities of other, more distantly related, organisms. All the Studies thus far published have dealt more or less with chemotaxis, and in concluding the present paper upon that subject, it will be well to summarize in the form of brief statements the important conclusions in regard to the phenomena which have gone under this name, drawing upon the previous Studies as well as the present one for this purpose and emphasizing some important points which have not been emphasized elsewhere.

1. The motor reaction by means of which chemotaxis is brought about is identical with that produced by mechanical shock or any other stimulus, so that chemotaxis is not an activity differing *in kind* from others. This motor reaction is as follows: the animal swims backward, turns toward one side which is structurally defined (the aboral in Paramecium), then swims forward.

2. This motor reaction is the same, whatever the position of the chemical or other stimulus, or the part of the body of the animal that it acts upon, so that the same chemical which when acting on the anterior end of the animal causes it to move away, causes it when acting upon the posterior end to approach. The direction of motion has no relation to the localization of the stimulus. The usual relation of the direction of motion to the position of the stimulus depends upon the fact that owing to the forward motion, stimulation almost universally occurs at the anterior end.

3. Negative chemotaxis or repulsion toward any substance is due merely to the fact that this substance sets in operation the one motor reaction above described. The Paramecium then does not come to rest until the random movements thus caused have carried it by chance, as they must in time, into a region containing none of the substance which causes the motor reaction.

4. Different chemicals have different specific effects upon the various component parts of the above reaction; one part may be intensified or weakened, while other parts are modified in some different way. The motion to or from the chemical is the resultant of these specific effects taken together. The direction of motion, known as positive or negative chemotaxis, is therefore a complex phenomenon, resulting indirectly from various factors.

5. Paramecia are nearest the quiet condition when in a weakly acid solution. Paramecia at rest under normal conditions are in such

a weakly acid solution, due to the carbon dioxide in the water, produced by themselves.

6. When Paramecia in such a weakly acid solution come in contact with a solution containing no acid, the motor reaction (with its reversal of cilia) is produced, so that the Paramecia do not pass out of the acid solution.

7. On passing from a neutral solution to a weakly acid solution no reaction is caused, but if now the Paramecium attempts to pass back again out of the acid solution, the reaction is produced (as in 6), so that the animal remains in the acid solution. The acid thus changes the physiological condition of the Paramecium, so that it now reacts to the neutral fluid.

8. The anions or acid ions present in salts tend to throw the Paramecia into the same physiological condition as the pure acid, so that the Paramecia afterward react when they come in contact with a fluid not containing these ions.

9. It results from 5, 6, 7, and 8 that Paramecia collect in solutions having a weakly acid reaction, or in solutions of salts in which the anion is especially active. Thus the so-called positive chemotaxis of Paramecium is brought about. The positive chemotaxis toward certain nutritive fluids, such as meat extract, is due to the acid reaction of the latter, not to its nutritive value.

10. Alkaline solutions and solutions of salts containing the kations of the alkali metals or of the earth alkali metals produce in a marked degree the motor reactions of Paramecium. As a result the Paramecia do not come to rest until their random movements have brought them into a solution which does not contain a large proportion of these ions. Thus the apparently negative chemotaxis of Paramecia toward these substances is brought about. In a series of salts of any one of the halogens with the different alkali metals, the power of producing the motor reaction (and hence the repellent power) decreases as the atomic weight increases, *except* that potassium salts are throughout more repellent than the lighter sodium salts.

11. In solutions of salts containing the kation of a strong alkali metal, as well as a powerful anion, as of fluorine or chlorine, both of the ions may act on the Paramecia, — the former causing the motor reaction when the Paramecium attempts to enter the solution, the latter when it attempts to leave it. In this case the Paramecia gather in a ring in the outer margin of a drop of the solution and are apparently both repelled and attracted by it.

12. The chief factor causing the motor reaction which results in negative chemotaxis is not the injuriousness of the substance, but is of a chemical nature. Two substances of equally injurious properties have by no means equal repellent powers, but those which are alkaline or contain kations of the alkali or earth alkali elements are much more repellent than most others, without regard to injurious effects.

13. Nevertheless, any substance which directly and severely injures the Paramecia does thereby cause a motor reaction. But this reaction is not produced till the injury has occurred and it is too late to save the Paramecia. They therefore swarm into chemical substances of certain sorts by which they are immediately killed.

14. It is therefore possible to distinguish (1) reactions due purely to the chemical nature of the substance, the reacting Paramecia being entirely unharmed, and (2) reactions due purely to the injury produced by the substance, without regard to its chemical nature. Reactions of the latter class are not sufficiently precise to be protective.

The investigation of which the results are presented in the foregoing paper was pursued in the Laboratory of the United States Fish Commission for the Biological Survey of the Great Lakes, at Put-in-Bay, Ohio, in the summer of 1898, and continued at Dartmouth College during the following autumn. I desire to express my sincere thanks to the officials of the U. S. Fish Commission and to Professor J. E. Reighard, Director of the Survey, for courtesy and assistance in every way during the progress of the work.

THE CHEMISTRY OF THE MELANINS.

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Introductory.

THE name "melanin" is a generic term which is used to include all of the dark brown or black animal pigments, whether formed in the body by normal processes, or under pathological conditions. Owing in a measure to the great interest which these substances possess for the pathologist they have been the subject of a number of researches of a chemical nature, yet for some reason these researches have been so fruitless that at the present time we are not in a position even to define a melanin in a chemical sense; in fact, we are not all agreed as to what chemical elements are necessary constituents of a melanin molecule. If we consider for a moment the methods which have been employed for the isolation and purification of the pigments, and at the same time grant that these substances may be very sensitive to the action of chemical reagents, and that it is also within the bounds of possibility that the composition of the pigment, like that of hæmoglobin, is different for different animal species, we will then be in a position to appreciate just such a discordance of analytical results as that which actually exists.

Since these substances have been looked upon as very stable organic compounds, the general method which has been employed for their isolation from the tissues consists in submitting the pigmented material to such chemical processes as will decompose or remove adherent impurities, on the assumption that the stable pigment will remain unaltered by the reagents that have been used. This has been variously accomplished by the action of acids or alkalies, by artificial gastric digestion,[1] and by putrefaction.[2] The residual pigmented material was formerly submitted to various purifying processes, or analyzed without further purification in accordance with the experimenter's attitude towards the prevailing view that the pigments are derived from the

[1] Brandl and Pfeiffer: Zeitschrift für Biologie, 1890, xxvi, p. 348.

[2] Dressler: Prager Vierteljahrschrift, 1866, iv, p. 9. (Quoted from Archiv f. exper. Pathol. u. Pharmakol., 1897, xxxix, p. 79.)

non-proteid part of the red coloring matter of the blood, and the consequent expectation of showing the presence or absence of iron in the product obtained.

Three years ago Abel and Davis[1] showed that the coloring matter of the negro's skin and hair consists of a colorless ground substance which is stained with the pigment, and that in order to remove the pigment from this ground substance a certain definite order of chemical procedure is absolutely necessary. Since these conditions have not been met by most experimenters it seems highly probable that many of the pigments hitherto described are not true pigments, but so-called granules, and that the supposed solutions of pigments in alkalies were only suspensions in such a fine state of division as to pass easily through filter papers. In fact, one who is acquainted with pseudo-solutions of pigmented granules can now easily understand from the descriptions in the literature that many of these so-called solutions of pigment were not solutions at all.

This discovery, however, does not bring about the desired conformity in analytical results, for Abel and Davis found that the composition of their true pigments is easily subject to change by subsequent treatment of the material with alkalies. Schmiedeberg,[2] also, who was perfectly familiar with the distinction between granules and true pigment, found that the pigment acid obtained by two slightly different processes from a melanotic sarcoma differed so widely in chemical composition as to be represented by such fundamental formulas as these,

α-Sarco-melaninic acid, $C_{68}H_{65}N_{11}SO_{29} \cdot 10\frac{1}{2}\ H_2O$.
β- " " " $C_{68}H_{67}N_{13}SO_{25} \cdot 3\frac{1}{2}\ H_2O$.

In fact, it seems that the material called a melaninic acid is even more sensitive to treatment with chemical reagents than the pigmented granules themselves. For this variation in the composition of a given melanin two causes may be assigned.

First. The material called a melanin may be a mechanical mixture of the pigment with some impurity, the latter being gradually removed by the action of chemical reagents.

Second. The melanin may be a substance whose molecule is very sensitive, and easily changes its composition in the process of chemical manipulation.

[1] Abel and Davis: Journal of experimental medicine, 1896, i, p. 381.

[2] Schmiedeberg: Archiv f. exper. Pathol. u. Pharmakol., 1897, xxxix, p. 77.

On either assumption it would be interesting to know how far this change in composition can proceed, and for the purpose of investigating this question the work which is about to be described was undertaken.

Preparation of Melanin Granules.

Black hair from the tail of the horse having been carefully sorted and cleansed by washing with water, was closely packed in glass jars and then treated with concentrated hydrochloric acid[1] in great excess. From time to time the contents of the jars were stirred, and as the material became disintegrated successive additions were made until 450 grams of hair had been used for every litre of acid. After the final addition of hair the contents of the jars were allowed to stand, with occasional stirring, during the period included between August 1 and October 7. After the acid had thus acted for ten weeks at the summer temperature the hair was found to be completely disintegrated, the product consisting of a dark brown liquid beneath which was a muddy gelatinous mass, there being no sharp line of demarcation between the two. This material was treated with an equal volume of tap-water, and as subsidence had occurred fairly well after standing over night, the muddy liquid was siphoned from the black sediment. A large volume of tap-water was then added and the vessel thoroughly shaken. As subsidence did not occur after several days, enough caustic soda was added to give the liquid a decided alkaline reaction, and the material was then made faintly acid with hydrochloric acid. Under these conditions the granules settle immediately, leaving a perfectly colorless liquid which can be sharply siphoned off. A large volume of tap-water was again added, and this process of washing by decantation was repeated many times, the alkaline fluid being occasionally filtered through coarse filter-paper, and the last washings made with distilled water instead of tap-water.

The soluble proteids being thus removed, alcohol was substituted for water and the washing by decantation continued, the alcohol being made alternately alkaline and acid, as in the case of washing with water. After the last portion of alcohol had been siphoned off, the muddy mass of granules was placed on filters and washed successively with absolute alcohol and ether. Experience has shown that it is not advisable to use a filter-pump for this or any filtration which will be mentioned in this paper. The liquid will run continuously for many

[1] Abel and Davis: *loc. cit.*, p. 369.

hours, through a plain filter, and while the operation seems quite slow at the beginning, it does not become any slower as the filtration proceeds. In case an analysis of the granules is required, material can be taken with a spatula from the centre of the filter without fear of contamination by fibres of paper.

When a portion of the ether washings left only a slight residue on evaporation, the filters were allowed to stand uncovered until most of the ether had evaporated and then placed in desiccators provided with sulphuric acid and shavings of paraffin. When the material on the filters begins to show cracks it can be removed easily, but after it becomes perfectly dry there is danger of removing filter fibres. The thick paste of granules was ground to a powder in an agate mortar and thoroughly dried in a desiccator over sulphuric acid.

A small weighed portion of this material was heated to constancy of weight at 110° C. From the numbers thus obtained with the known weight of desiccator dry granules obtained from a certain weight of hair, it was found that the dry granules constitute about 5½ per cent of the hair in its normal condition. In the case of the negro's hair Abel and Davis found the proportion of granules to be less than 2 per cent.

Preparation of Melaninic Acid from Melanin Granules.

One hundred grams of caustic potash were melted in a nickel bowl with 10 c.c. of water, and 10 grams of granules were added in small successive portions, the material being continually stirred and a cubic centimetre of water being added from time to time. Immediately after the addition of a portion of granules there was considerable smoking with a decided odor of burnt hair, but the material very soon came into a state of calm fusion. After the last addition of granules, heating was continued for about ten minutes at temperatures which were near 350° C., and the melted mass was finally poured into cold water and allowed to stand in a porcelain bowl until the solution was perfectly cool. The fluid, which possessed a most intense indol odor, was diluted with water to a volume of 1000 c.c. and filtered through hardened filters. On diluting a small portion of the black filtrate with water there was obtained a perfectly transparent Bordeaux red liquid which was immediately recognized as a true solution. The alkaline filtrate was made faintly acid with hydrochloric acid, and after standing twenty-four hours the pale yellow liquid was siphoned from the melaninic acid that had fallen to the bottom. The latter was thrown on

filters, washed with hot water for several hours, and spurted from the filters into tall glass jars by means of a jet of hot water. The material was then alternately dissolved in large volumes of extremely dilute alkali, and precipitated by the addition of a trace of hydrochloric acid, the last alkaline solution having been filtered to remove particles of dust and fibres of filter-paper. When alkali is added the melaninic acid goes sharply into solution, and on acidifying such a solution with hydrochloric acid the pigment falls out as a reddish brown flocculent precipitate which settles immediately, leaving a perfectly colorless liquid above. A check test was made to show that ten times the approximate percentage of sodium chloride present could not be precipitated by the addition of any amount of absolute alcohol. As this test gave the desired result, the melaninic acid was washed by decantation with absolute alcohol until the water was removed, when ether was substituted and the washing by decantation continued. The subsequent treatment of the material was the same as that described above for the purification of the granules, except that a large quantity of the melaninic acid was removed from the centre of the filters, ground dry of ether in an agate mortar, and reserved for analysis.

The product thus obtained is a dark brown amorphous powder with a yellowish brown streak which causes the substance to appear lighter in color when it is ground in a mortar. It is insoluble in alcohol, ether, benzene, acetic ether, and chloroform, but quite easily soluble in distilled water, giving to the latter a decided acid reaction to litmus. From its solution in water it is precipitated by dilute hydrochloric or sulphuric acid, but not by acetic acid. When dried for a long time in a desiccator over sulphuric acid the pigment loses its solubility in water, but is soluble in water that contains a trace of sodium hydroxide, forming a fine, transparent red solution. When such a solution is diluted and made acid, a light yellow turbidity occurs, which gradually turns darker and finally collects into reddish brown flocks, leaving a perfectly transparent liquid. The alkaline solution is easily decolorized by passing in chlorine or by boiling with potassium permanganate in proper quantity. When dried at 110° the pigment becomes very difficultly soluble in alkalies. On heating in a tube over a free flame the melaninic acid develops a very offensive indol odor, a colorless deposit occurs on the colder part of the tube, and vapors are given off which yield an intense pyrrol reaction with a pine splinter that has been moistened with concentrated hydrochloric acid. There is, however, no odor of burnt hair produced.

The Composition of Melaninic Acid.

A quantity of material which was found on microscopic examination to be free from fibres of filter-paper was dried in small portions to a constancy of weight at 110° and analyzed.

In a silver crucible was fused 0.2883 gram of the substance with caustic potash and potassium nitrate for the purpose of making a quantitative determination of sulphur. The cake was dissolved in water, the solution made acid with hydrochloric acid and filtered from a trace of silver chloride. On heating the filtrate and adding barium chloride not the slightest clouding occurred in the course of half an hour, yet the addition of a trace of sulphuric acid produced an immediate precipitate.

I. 0.1823 gram substance gave 0.0008 gram ash = 0.44 per cent.

II. 0.2801 gram substance gave 31.5 c.c. moist nitrogen at 15.5° C. and under a barometric pressure of 762 mm.

III. 0.1856 gram substance gave 0.3939 gram CO_2 and 0.0586 gram H_2O.

Part of the same material, before treating with alcohol and ether, was precipitated additionally six times from a dilute alkaline solution with hydrochloric acid. The alkaline solutions were left freely exposed to the air, and the final product, dried to a constancy of weight at 110°, gave the following analytical results:—

IV. 0.2001 gram substance gave 0.0009 gram ash = 0.45 per cent.

V. 0.2355 gram substance gave 27 c.c. moist nitrogen at 22.5° C. and under a barometric pressure of 763 mm.

VI. 0.1861 gram substance gave 0.3937 gram CO_2 and 0.0568 gram H_2O.

	Percentage composition of ash-free substance.				*Theoretical for*
	II	III	V	VI	$C_{56}H_{36}N_{10}O_{17}$
C	....	58.14	....	57.94	58.20
H	....	3.52	....	3.41	3.36
N	13.18	....	13.07	....	13.06

While the properties of this substance which have been noted are at once recognized as characteristics of the melaninic acids, it might nevertheless be objected that the rather rough treatment to which the material has been subjected is well calculated to so alter its chemical constitution that it bears only a remote chemical resemblance to the melanin that actually exists in the body. As so little is known, however, of the chemical relation between living and dead matter, it seems difficult to decide what chemical processes are to be regarded as permissible for the isolation of a substance from the

tissues. The objection offered may therefore with equal propriety be applied to almost every preparation known to physiological chemistry, for what is regarded as very mild treatment to some substances would be most severe when applied to others. It being universally granted that a chemical change is always accompanied by a change of properties, it would seem that we are more concerned with the characters of the substance in question than with the methods by which it has been obtained.

While we believe that the pigment is not identical with that originally present in horse hair we fail to see any sufficient evidence for regarding the former as a product of deep-seated chemical action.

Nor does the absence of sulphur from this material constitute any argument against its acceptance as a true melaninic acid, for Sieber[1] obtained from the choroid coat a sulphur-free pigment, having subjected the material to no more severe an operation than boiling for two hours with ten per cent hydrochloric acid. Later, Hirschfeld[2] obtained a similar result by using an acid of a strength less than five per cent and working in the cold, — a precaution which was employed to avoid the production of artificial melanins, which are liable to be formed by the action of stronger acids on adherent proteids.[3] From this point of view one might think of the pigment that has been described in this paper as an artificial choroid pigment. Such, however, is not the case, for Hirschfeld[4] has shown that when the choroid pigment is treated with melted alkalies, a product is obtained which is free from nitrogen.

The literature shows still another instance of a melaninic acid obtained by melting a natural product with alkalies, namely, the hippomelaninic acid of Nencki and Sieber.[5] Although this substance resulted from a longer treatment with alkali than the one which we prepared, two preparations were found to contain 1.39 per cent and 2.6 per cent of sulphur respectively. Moreover, it is distinguished from the melaninic acid of Hirschfeld in its nitrogen content (10.41 per cent). It therefore seems clear that whether these products are to be regarded as chemically altered pigments or not, their marked chemical differences from one another constitute a conclusive proof

[1] Sieber: Archiv f. exper. Pathol. u. Pharmakol., 1886, xx, p. 363.

[2] Hirschfeld: Zeitschr. f. physiol. Chemie, 1889, xiii, p. 418.

[3] See Schmiedeberg: Archiv f. exper. Pathol. u. Pharmakol., 1897, xxix, p. 70; also Chittenden and Albro: This journal, 1899, ii, p. 291.

[4] Hirschfeld: *loc. cit.*, p. 430

[5] Nencki and Sieber: Archiv f. exper. Pathol. u. Pharmakol., 1888, xxiv, p. 17.

that the three physiological pigments which they represent must themselves have corresponding differences. This involves the assumption that in at least one instance the pathological pigment (Nencki's hippomelaninic acid) is different from the normal pigment (melaninic acid from horse's tail) in the same animal species.

An extension of this work is now being carried on in this laboratory which will include other natural pigments as well as artificial melanoid bodies formed by the action of acids on various proteids.

Oxidation of Melaninic Acid. Oxymelaninic Acid.

(*a*) **Oxidation in an alkaline medium.** — The following preliminary experiments were made with the melaninic acid from the negro's hair, the process employed for the isolation of the pigment being essentially that described above for the preparation of the corresponding substance from the horse's tail.

A quantity of melaninic acid was dissolved in water which contained enough sodium hydroxide to effect an easy solution of the pigment. As this solution was boiled, successive small portions of a faintly alkaline solution of potassium permanganate were added until the clear liquid between the particles of precipitated manganese dioxide hydrate showed by its faint pink color that the permanganate was in slight excess. A sufficient amount of a solution of oxalic acid was then added to reduce the excess of permanganate and dissolve the precipitate. The solution had a pale yellow color due in all probability to the presence of a slight amount of pigment that had become included in the precipitated manganese dioxide hydrate and had thereby escaped oxidation. The oxalic acid and manganese were removed in the ordinary way with barium hydroxide, and the excess of the latter was precipitated by passing carbon dioxide into the hot solution. The filtered liquid was evaporated to dryness on the water-bath and the small quantity of pale yellow residue was examined. Aside from a very unsatisfactory reaction for pyrrol with a pine stick moistened with hydrochloric acid, and a slight gray charring when heated on the foil, no organic matter could be detected. The various precipitates were also examined for organic carbon with negative results.

Similar experiments were also made with larger quantities of material, using ammonium permanganate as the oxidizing agent, and working at temperatures between 0° and 5° C. Even under these conditions the permanganate is almost immediately decolorized, showing that in alkaline solution the pigment is oxidized with the greatest ease. In

this instance organic matter could undoubtedly be proved among the products of the reaction, but its amount was so insignificant that its presence is probably referable to undecomposed pigment.

As it is quite possible that volatile substances formed in these oxidations may have escaped in the manipulation, the following experiment was made in such a manner as to preclude the possibility of any volatile acid or basic substance being lost. About two grams of melaninic acid from the horse's tail were dissolved in a considerable excess of sodium hydroxide, and a gentle stream of chlorine gas was passed into the alkaline solution. For the purpose of retaining volatile basic substances, the end of the apparatus was provided with an absorption bulb which contained more than half enough hydrochloric acid to neutralize the sodium hydroxide in which the pigment was dissolved. The pigment becomes rapidly decolorized, and in a few minutes a solution results which is no more highly colored than a solution of chlorine gas in water. This material was divided into two equal portions, and after adding to one of these the hydrochloric acid solution contained in the terminal bulb, both portions were evaporated to dryness on the water-bath and the residues examined. That which resulted from the acid portion was found to contain a large quantity of an ammonium salt, but neither residue gave the slightest evidence of organic carbon. A similar experiment with the melaninic acid from the negro's hair gave the same result. The conclusion, therefore, seems to be justified that these pigments are easily oxidized in alkaline solution, and that in all probability the oxidation is so complete as to give rise to such simple substances as carbon dioxide, water, and ammonia.

(*b*) **Oxidation in an acid medium.** — When a solution of the pigment in water is treated with chlorine, the result is quite different from that obtained by using an alkaline solution. In the earlier stages of the oxidation the pigment is thrown down in consequence of the acidification of the solution; but its color soon begins to depart, and in the course of half an hour is light reddish-yellow. A continuation of chlorine has now but little effect, for it was found impossible in a trial experiment to completely decolorize a gram of pigment by treatment with chlorine for eight hours. Several portions of pigment which were roughly estimated to aggregate ten grams were treated with chlorine in tall cylinders and the reddish-yellow emulsions were united and warmed on the water-bath. The undissolved material soon became flocculent, leaving a clear, almost colorless supernatant

liquid. The solution was filtered off and the pigment, which had become somewhat darker, was spurted into a tall cylinder, and alternately dissolved in sodium hydroxide and precipitated with hydrochloric acid. At each step of this procedure the material was found to be darker in color until a product was finally obtained which in its general appearance is indistinguishable from the melaninic acid originally used for the oxidation. The acid solution was finally siphoned off and the pigment washed by decantation with successive large portions of absolute alcohol. Ether was then substituted for alcohol, and the pigment was finally obtained as a dry dark brown powder by a process similar to that described above for the preparation of melaninic acid. The product dried in a desiccator over sulphuric acid is soluble in water but insoluble in organic solvents. When heated it gives off a very strong skatol odor and produces fumes which impart an intense red color to a pine stick that has been moistened with hydrochloric acid. In fact there is nothing connected with its physical properties or chemical reactions which would suggest that the substance is any other than the melaninic acid from which it was prepared. Having determined the absence of chlorine, the substance was dried to a constant weight at 110° and analyzed.

I. 0.2242 gram substance, after fusion with potassium nitrate and potassium hydroxide, gave no precipitate in acid solution with barium chloride.

II. 0.0914 gram substance gave 0.0006 gram ash = 0.66 per cent.

III. 0.2092 gram substance gave 0.3625 gram CO_2 and 0.0474 gram H_2O.

IV. 0.1770 gram substance gave 0.0382 gram H_2O.

V. 0.1796 gram substance gave 24.1 c.c. of moist nitrogen at 18.5° C. and under an atmospheric pressure of 759.5 mm.

	Percentage composition of the ash-free substance.			*Theoretical for*
	III	IV	V	$C_{18}H_{11}N_3O_{10}$
C	47.60	....	....	47.26
H	2.53	2.42	....	2.41
N	....	....	15.56	15.32

The formation of this substance, which may be called oxymelaninic acid, must therefore be expressed by the following equation :

$$C_{52}H_{36}N_{10}O_{17} + 42\,O = 2\,C_{18}H_{11}N_3O_{10} + 16\,CO_2 + 7\,H_2O.$$

Attention was next given to the soluble products formed by the action of chlorine on melaninic acid in acid solution. The liquid containing these substances was light yellow in color, but became gradually darker as evaporation on the water-bath continued, reaching finally a very dark red and depositing flocks of a brown material

which is in all probability identical with oxymelaninic acid. The precipitate was filtered off and a drop of the filtrate was made alkaline with sodium hydroxide. A volatile base was given off which fumed in the air, changed red litmus, and gave dense white vapors with a drop of dilute hydrochloric acid held upon a stirring rod. This base has a very characteristic odor, which strongly resembles that of semen, but the odor of ammonia could not be detected. Suspecting the base to be a substance closely related to if not identical with putrescine, a large portion of the solution was made alkaline with sodium hydroxide and distilled, the distillate being received in a small quantity of dilute hydrochloric acid. The acid distillate was evaporated on the water-bath, platinum chloride was added, and the precipitated platinum compound was recrystallized from hot water and analyzed.

I. 0.2177 gram substance gave 0.0923 gram platinum.
II. 0.2365 gram substance gave 0.1027 gram platinum.

	Theoretical for $(NH_4)_2PtCl_6$	*Found.* I	II
Pt	43.79	43.60	43.43

So large a quantity of this substance (which is undoubtedly ammonium platinum chloride) was obtained that we must look upon its precursor as the principal nitrogenous product of the oxidation. An analysis of the salt as it was precipitated by platinum chloride without crystallization from hot water gave 39.89 per cent of platinum. This would indicate that with the ammonium platinum chloride there is also precipitated a more soluble platinum compound which contains less platinum than does ammonium platinum chloride.

A comparatively large quantity of the original acid solution was made alkaline with sodium hydroxide and allowed to stand over night under a bell-jar with a small quantity of very dilute sulphuric acid. On removing the bell-jar there was a most pronounced odor of ammonia, although no such odor could be detected immediately after the solution had been made alkaline. The drop of sulphuric acid was diluted with a little water and various alkaloidal reagents applied, but with negative results. Unsuccessful attempts were also made to shake out the base from an alkaline solution with chloroform and ether.

Part of the original acid solution was treated with benzoyl chloride and sodium hydroxide. The liquid was shaken out with ether, and after the ether had been evaporated the residue was boiled with

hydrochloric acid. The benzoyl compound is very resistant but finally goes into solution. On making this solution alkaline with sodium hydroxide an odor like that of putrescine can be detected. If this substance is related to putrescine it is probably one of its numerous isomers, which is far more sensitive to the action of alkalies than putrescine itself. Although the base is formed in relatively large quantity by the action of chlorine in acid media on the melaninic acids from the horse's tail, from human melanotic sarcoma, and from the negro's hair, no method has yet been found for its isolation from the products of the reaction. We believe that the substance can be identified by an analysis of its benzoyl compound, but it is apparent from what has already been stated that to obtain the base itself some very special method of manipulation must be used.

Conclusions.

(1) An easy and convenient method for the preparation of the pigmented granules in any desired quantity from hair consists in treating sorted hair with concentrated hydrochloric acid for a long time at ordinary temperatures.

(2) It is possible to prepare from these granules two substances that must be regarded as melaninic acids, namely, melaninic acid and oxymelaninic acid. Both of these substances are free from sulphur, and in this regard they do not differ from the pigment which both Sieber and Hirschfeld isolated from the choroid coat. One of these melaninic acids was shown not to change its composition by exposure of its solution in dilute alkalies to the oxidizing action of the air, and the other was prepared in such a manner as to suggest that it is not likely to undergo chemical change without deep-seated decomposition. The relation in composition between these two melaninic acids is expressed by the following equation: —

$$C_{52}H_{36}N_{10}O_{17} + 42\,O = 2\,C_{18}H_{11}N_5O_{10} + 16\,CO_2 + 7\,H_2O.$$

This reaction, which occurs under the influence of chlorine gas, strongly reminds us of the oxidation under similar circumstances of various organic compounds which contain several aromatic nuclei in combination, and which lose in the oxidation an approximately equal number of carbon and hydrogen atoms. The oxidation of naphthalene by means of chlorine to form phthalic acid may serve as an instance.

$$C_{10}H_8 + 4\,O = C_8H_6O_4 + 2\,CO_2 + H_2O.$$

It is of interest to note the relation in chemical composition between the various pigments that have been obtained from the horse's tail. For this purpose the following formulas have been constructed: —

1. $C_{58}H_{68}N_{10}O_{16}$.
2. $C_{58}H_{59}N_{10}O_{18}$.
3. $C_{52}H_{31}N_{10}O_{17}$.
4. $C_{36}H_{22}N_{10}O_{20}$.

Formulas (1) and (2) represent two analyses of a pigment which was prepared by Nencki and Sieber.[1] From the analytical data given fundamental formulas have been constructed according to Schmiedeberg,[2] and from these formulas that for sulphuretted hydrogen has been subtracted. The expressions thus obtained were divided by such a quantity as would bring the number of nitrogen atoms to ten. The formulas show progressive oxidation as we pass from (1) to (4). The last member of the series contains relatively more oxygen than any melaninic acid hitherto prepared, and has therefore been termed oxymelaninic acid.

(3) The sulphur-free pigments prepared by me are easily and completely oxidized in akaline solution even at temperatures that are very near 0° C.

(4) By treatment of one of these sulphur-free pigments with chlorine in an acid medium, a volatile basic substance is formed which has an odor very similar to that of putrescine, is easily decomposed by treatment with alkalies leading to the formation of a large amount of ammonia, and yields a benzoyl derivative from which the base can be regenerated. The production of this base from melanins forms part of a chain of evidence to show a chemical relation between these pigments and proteids. E. Schulze and Winterstein[3] have obtained ornithin by hydrolysis of arginine with barium hydroxide, and Ellinger[4] has succeeded in isolating putrescine from the putrefactive products of ornithin. As arginine is a common hydrolytic product of all proteids, it follows that in the proteid molecule there exists some grouping of atoms which corresponds to putrescine and which is a necessary part of the proteid molecule. The discovery of a base, therefore, which closely resembles putrescine in its physical and chemical properties, and which is a common decomposition product

[1] Nencki and Sieber: Archiv f. exper. Pathol. u. Pharmakol., 1888, xxiv, p. 20.

[2] Schmiedeberg: *Ibid.*, 1897, xxxix, p. 2.

[3] Schulze, E., and Winterstein: Berichte der deutschen chemischen Gesellschaft, 1897, xxx, p. 2879.

[4] Ellinger: *Ibid.*, 1899, xxxii, p. 3183.

of three melaninic acids, suggests that some similar grouping exists in the melanin molecule. This conclusion is also in conformity with the results which Nencki[1] obtained by a study of the products of artificial pancreatic digestion. He has shown that the bromine derivative of tryptophan consists of at least two substances, one of which is a brown pigment whose composition (reckoned bromine free) is almost identical with that of hippomelaninic acid.

It is not my purpose to offer in this paper any theory of my own as to the chemical processes by which the normal or the pathological pigments are derived from proteids. Nencki[2] holds that the proteinchromogens formed during pancreatic digestion may well be the mother substance of all the animal pigments. Schmiedeberg,[3] as already stated, has prepared artificial melanins from proteids, and has given an outline in a broad and masterful way of the possible chemical processes involved in the derivation of the natural pigments from their precursors. Very recently Nencki[4] has remarked that Schmiedeberg's artificial melanins are apparently derivatives of proteinchromogen. Chittenden and Albro[5] have recently verified and extended Schmiedeberg's observations, and have shown that antialbumid and hemipeptone can yield artificial melanins whose percentage composition enables a comparison to be made, in this respect at least, with the natural melanins. Much work of an exact chemical nature still remains to be done before we can make any very definite statements as to the manner in which the natural pigments are formed from their proteid precursors.

[1] NENCKI: *Ibid.*, 1895, xxviii, p. 560.

[2] NENCKI: *loc. cit.*, p. 566.

[3] SCHMIEDEBERG: *loc. cit.*

[4] NENCKI: Footnote to Loew's abstract of Schmiedeberg's paper in Jahresber. ü. d. Fortschr. d. Thier-Chemie, 1896, xxvii, p. 13.

[5] CHITTENDEN and ALBRO: This journal, 1899, ii, p. 291.